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GENETIC ASSIMILATION OF THE *BITHORAX* PHENOTYPE

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INTRODUCTION

Some years ago it was suggested (Waddington, 1942) that if selection was practised for the readiness of a strain of organisms to respond to an environmental stimulus in a particular manner, genotypes might eventually be produced which would develop into the favoured phenotype even in the absence of the environmental stimulus. A character which had originally been an "acquired" one might then be said to have become genetically assimilated. An experimental investigation of the suggestion was carried out on *Drosophila melanogaster*, using a heat shock applied to the pupa as the environmental stimulus, and a crossveinless phenotype as the modification for which selection was practised. As has been described earlier (Waddington, 1953a), a genetic assimilation of the crossveinless phenotype was achieved in this case.

It seems possible that considerable general importance should be attached to processes of this kind, which appear able to provide a satisfactory explanation of the evolution of certain types of adaptation which have in the past been difficult to explain convincingly. It was therefore thought desirable to investigate the genetic assimilation of other characters, so as to broaden the observational basis on which the theory rests. Mrs. K. G. Bateman in this laboratory has studied a number of rather mild developmental modifications, produced by temperature shocks applied to the pupa. These can perhaps be considered as of the same general type as the crossveinless phenotype previously investigated. The present communication deals with experiments on a phenocopy of a rather different character. This is the bithorax-like modification which can be produced by ether treatment of the young embryo (Gloor, 1947). The phenotype involves a profound modification of the normal appearance of the animal. The meta-thoracic disc, which normally gives rise to the halteres, becomes changed so as to develop into structures resembling the normal mesothorax, including the wings. If such a change occurred during phylogenesis it would certainly be accounted a macro-evolutionary phenomenon. It was felt that, if such a fundamental modification as this can be genetically assimilated, then one would have some grounds for confidence that the process was powerful enough to be invoked to explain quite far-reaching evolutionary changes.

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Materials and Methods

The stock used was a mass bred Oregon *K* wild type. Eggs were collected over a one-hour period. At the age of 2½–3½ hours after laying they were given an ether vapour treatment by inverting the watch glass on which they were laid over an open dish of ether. The duration of the treatment was normally 25 minutes. The ether-treated eggs were allowed to hatch and develop into adults, all cultures being carried on at 25° C. On emergence, which was spread over a period of about two days, the flies were classified as wild type or bithorax phenocopies. In the up selection lines from the latter category sufficient males and females (virginity uncertain) were collected every 24 hours to produce the next generation of eggs. In most generations between 2,000–3,000 flies were classified, and to produce these several hundred parents were used in each generation. Parallel with the upward selection line in which the bithorax phenocopies were used as parents, a downward selection line was carried on, in which the parents were taken from the wild type flies. Two replicates were made of this two-way selection experiment, so that there were eventually four lines, i.e. Experiment 1, Upward Selection and Downward Selection, and Experiment 2, Upward Selection and Downward Selection.

Results

The two replicates were drawn from different initial stocks of Oregon R, and they started by showing rather different levels of phenocopy production. In Experiment I the first generation gave 24.5% of bithorax phenocopies, whereas Experiment II gave 48.8%. From these two initial points both the upward and the downward selections were effective. There

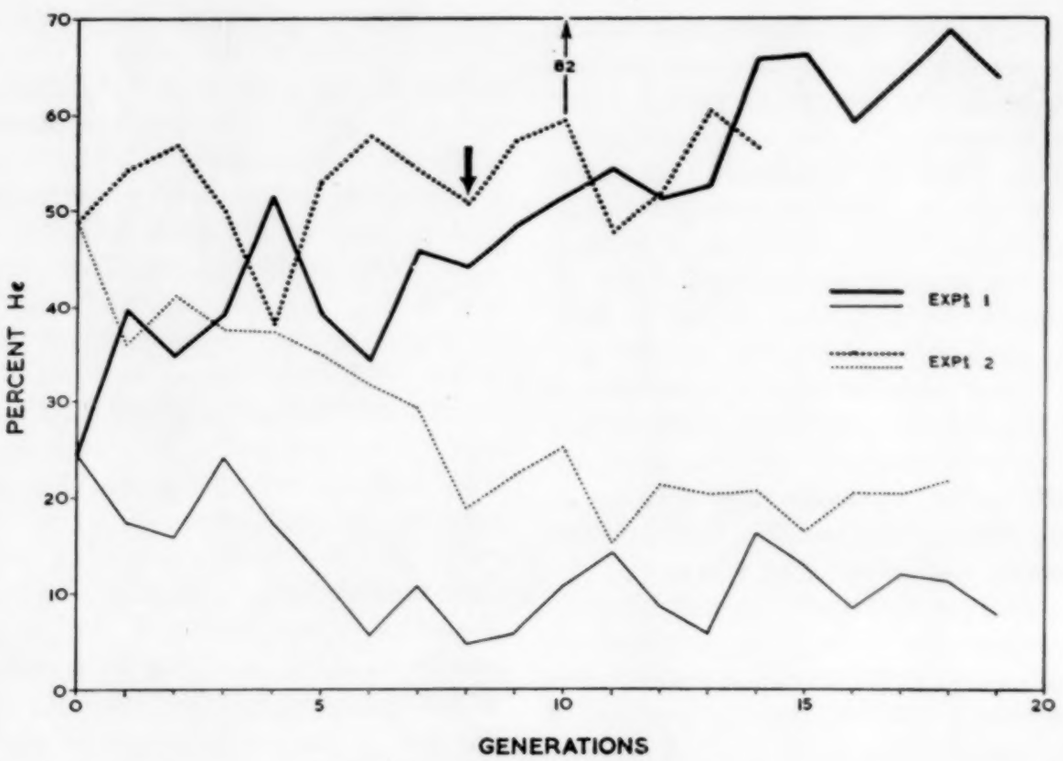

Fig. 1. Percentage of He (= bithorax-like) phenocopies in successive generations of upward and downward selection, following ether treatment of the eggs. The figures relate to flies which emerged from the puparia. At generation 10 in Experiment II, a count was made of all flies in a sample, including those failing to emerge and a percentage of 82 He found. The arrow at gen. 8 in Experiment II indicates the point at which the first assimilated stocks appeared.

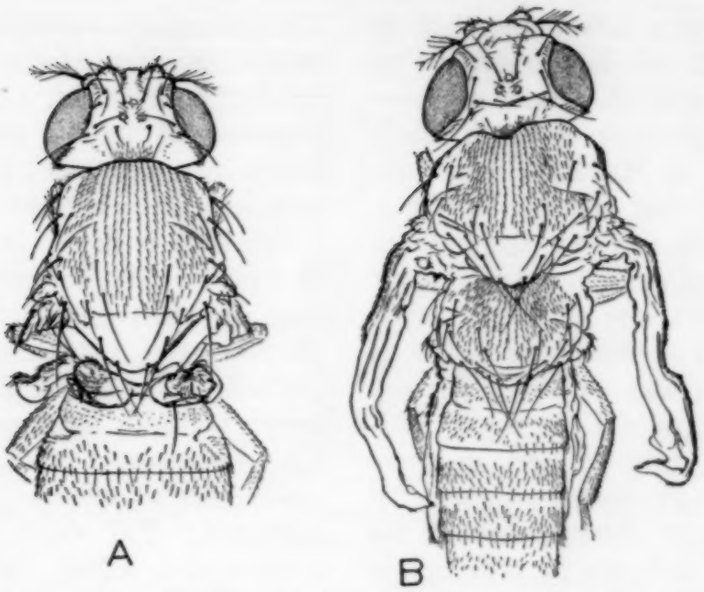

FIG. 2. Two typical phenocopies (medium and extreme in grade). Mesothoracic wings removed in 2a, metathoracic wings partly concealed by the abdomen in 2b.

is, however, not much point in quoting in detail the numbers of bithorax phenocopies which emerged in later generations, particularly in the upward selected lines. This is because a considerable proportion of high-grade phenocopies failed to emerge from the pupa cases. For instance, in generation 10 of Experiment II the proportion of phenocopies among the hatched individuals of the upward selection line was 59.5%, but from a sample of pupae in which the flies which failed to emerge were dissected out and examined, the proportion was about 82%. Normally such dissections were not done and the percentages of the phenocopies among the hatched adults, which are shown graphically in figure 1, give only a rough indication of the changes which the selection produced. It was also noted that in the upward selection lines the average grade of expression of the phenocopies rose, so that eventually a large number of very extreme bithorax types appeared (figure 2). It was in particular these most extreme types which had difficulty in emerging. The figures for the downward selection lines are probably more reliable, since in these, as the proportion of phenocopies was reduced, so also was the grade of expression, and there was a comparatively low incidence of failure of emergence. It will be noted that after many generations of downward selection the strains still produced a considerable and fluctuating number of phenocopies. A similar situation was found in the previous experiment with crossveinless (Waddington 1953a), where again selection did not succeed in rendering the stock completely resistant to the environmental stimulus.

The First "Assimilated" Stock

a. First set:

In each generation, besides the eggs which were collected over a timed interval for ether treatment, a number of others were collected and allowed to develop without treatment. In the early generations, as would be expected, no signs of bithorax-like phenotypes appeared, the flies being normal Oregon K wild types. However, in the eighth generation of the upward selection line of Experiment II, one fly from an untreated egg was found to show slightly enlarged halteres. In the next generation about 10 such flies were found among the untreated indi-

viduals. These were mated together in pairs and a number of "haltere-effect" or "He" lines were started. Each of these lines from the beginning threw a fairly large proportion of He flies, i.e. individuals with somewhat enlarged halteres. Selection was made amongst the lines to try to produce one in which the frequency of the haltere effect rose to 100%, or in which the phenotype was more extreme. In neither respect was success achieved. In the phenotype of an untreated individual it is very rare to find in these lines anything more extreme than the comparatively slight enlargement of the haltere illustrated in figure 3a, although in the first generation a few flies of slightly more extreme type appeared (fig. 3b).

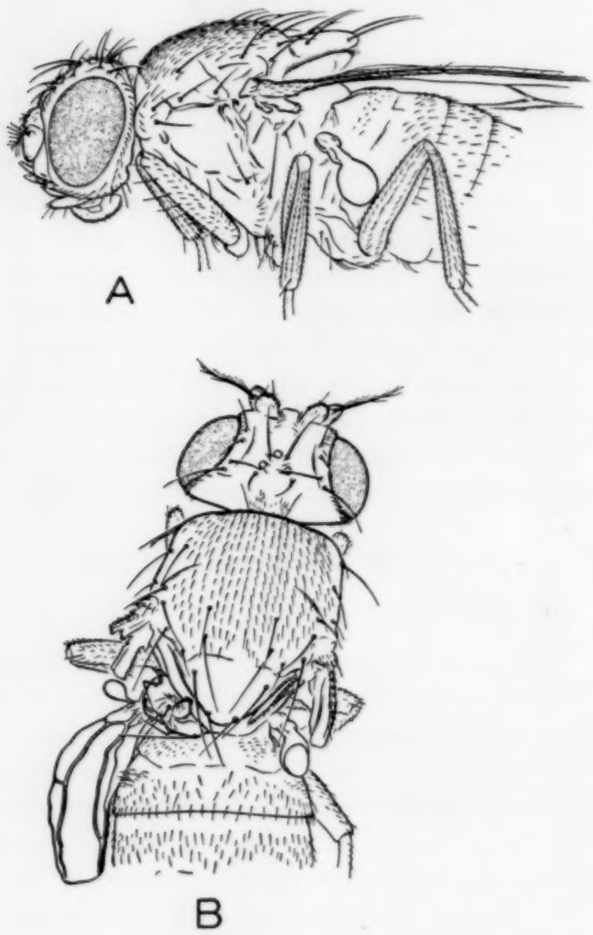

FIG. 3. Two individuals from the first set of "assimilated" stocks (He 17). Most flies of this stock show only the slightly enlarged halteres seen in 3a (cf. *Bxl*); a few have a bithoraxoid-like enlargement of the metathorax, as in 3b (from which the mesothoracic wings have been removed).

These were bithoraxoid-like; i.e. the metathorax represented the posterior part of the mesothorax (cf. Lewis, 1951). The frequency of the low grade phenotypes in one or two lines rose to 80% or slightly over, but never further.

Crosses with a *ClB*;Cy:Me stock showed that the haltere effect is due to a gene or genes on the third chromosome. In fact, the genetic condition can be perfectly balanced against Me, when it behaves exactly as though one were dealing with a single dominant gene with recessive lethal effect. Crosses to stocks containing Bxl show that the He/Bxl compound is lethal. All this is consistent with the hypothesis that the He effect is due to an allele of Bxl. The only thing which causes one any hesitation in adopting this hypothesis is the fact that in some of the "best" He stocks, and in their crosses with wild types, the numbers of He flies are higher than would be expected if He/He flies died. Thus in the selected He stocks, instead of the He individuals making up two-thirds of the progeny, they usually formed from 75% up to 85%; and in crosses and backcrosses with Oregon K and other wild types there was a consistent though not very large excess of He progeny (table 1). It was at first suspected that there might either be some polygenic tendency in the He stocks towards the enlargement of the haltere, cooperating with the major He gene, or that the lethality of the He homozygote was not absolute. Fairly extensive tests failed to confirm either suggestion. When the wild type individuals of an He strain were bred together no He's were ever produced in their progeny; nor could any He individual be found which gave rise to 100% He offspring. It was concluded therefore that the He effect is, in fact, produced by a single dominant with recessive lethal effect, but that, particularly in the strains which had been selected for a high proportion of expression, the viability of the heterozygote is somewhat greater than that of normal wild types.

TABLE 1. *Crosses of first He stocks*

	Parents ♀	Parents ♂		He's of 1st set (He 5–He 16) He	Total	He %	He's of 2nd set (He 17) He	Total	He %
1.	He	He	Gen. 1.	2639	3436	76.8	551	753	73.4
			Gen. 2.	1713	2232	76.7			
			Gen. 3.	871	1143	76.2			
2.	OrK	He		778	1476	52.7	406	822	49.4
2a.	He/OrK	He		2114	3072	68.8	702	1070	65.6
2b.	He	He/OrK					412	602	69.0
3.	He	OrK		748	1287	58*	389	767	50.6
3a.	He/OrK	He		1453	2026	72*	494	743	66.3
3b.	He	He/OrK					308	484	63.6

* The wild types used in these crosses were not OrK, but were from the downward selected line of Experiment II.

b. The second set:

These facts show that, in this first group of stocks in which the bithorax phenotypes seem to be to some extent assimilated, the "assimilation" has not proceeded in the normal way by the selection of many minor genes acting in the required direction, but occurred by the fixation of a single major gene mutation which presumably arose *de novo* by chance. Further selection for phenocopy production was therefore continued. Experiment II was carried to the 14th generation; Experiment I was taken to generation 20, when the duration of treatment was reduced to 20 minutes till generation 26, after which it was reduced again to 15 minutes. No further sign of assimilation occurred until generation 29 in the upward selected line of Experiment I. At this time a few untreated flies were found which showed indications of a bithorax-like phenotype. In most of these the abnormal phenotype was again confined to a slight enlargement of the halteres. By breeding these together and selecting amongst their progeny another haltere-effect stock known as Hel7 was produced. This behaved genetically exactly like the previously obtained haltere-effect stocks, the phenotype being due to a dominant with recessive lethal effect, which is indistinguishable from that previously described.

The Second "Assimilated" Stock

Among the untreated bithorax-like flies of generation 29 in Experiment I, one or two showed not only enlarged halteres but also extra pieces of thorax-like material formed on the dorsum by the metathoracic bud. These were bred together, and selection made amongst their offspring for the development of extra thorax. A few generations of such selection produced a stock known as He*,

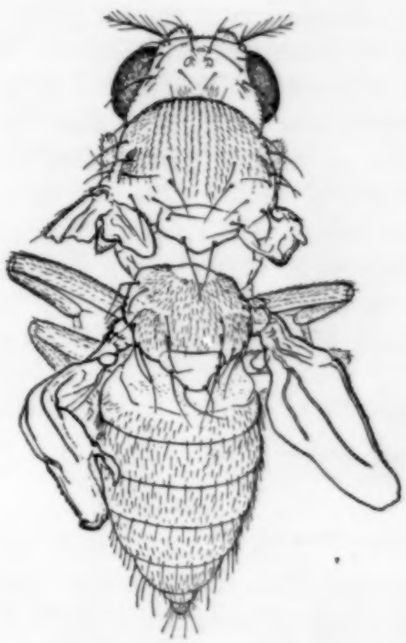

FIG. 4. An extreme, though fairly frequent, type from the second assimilated stock He*. Normal mesothoracic wings removed to show the bithorax-like metathorax.

which in mass matings gave a frequency of bithorax-like phenotypes of about 70–80%. These bithorax-like flies (fig. 4) frequently showed a large amount of extra thorax material as well as enlarged halteres. This extra material was of bithorax type, i.e. represented the anterior section of the mesothorax. The flies thus differed considerably in appearance from those of the previously described haltere-effect stocks. The difference persists even when eggs of the two stocks are given ether treatments and their phenotypic abnormalities thus exaggerated.

As a first step towards an analysis of the genetic situation in the He* stock a cross was made of an He* male to a ClB_1. Cy : Me female. No He* phenotypes appeared in the F_1. CyMe males and females from the F_1 were intercrossed and He* flies of rather weak grade were found in the F_2 in numbers which suggest that the character has a polygenic basis, the genes concerned being located both on the 2nd and 3rd chromosomes (table 2). There is no sign of any effect of recessive sexlinked factors.

This result falls in line with what one might expect if the assimilation were due to the accumulation of minor genes acting towards the bithorax phenotype. The results of crosses and back-crosses to wild types were, however, somewhat anomolous. The results of the first set of such crosses are given in table 3. It will be seen than when He* females were crossed with Oregon K males (of the stock used for Experiment II) there was a large percentage of He* individuals in the F_1,

TABLE 2. *C1B; Cy; Me* ♀ × *He** ♂ *gave no He** *phenotypes in F1.* $\frac{Cy;\ Me}{He^*}$ *mated* inter se *gave in F2:*

Offspring	He* ♀	He* ♂	non-He*	Total	He*%
+	31	23	106	160	33.7
Cy	24	31	179	234	23.5
Me	15	16	262	293	10.6
Cy; Me	15	8	506	529	4.3

TABLE 3. *He*: first set of crosses to OrK (1a, 1 bare back-crosses from 1; 2a, 2b from 2)*

	Parents ♀	Parents ♂	He*	Total	He*%
	He*	He*			70–80
	Non-He* ♀ and ♂ from He* stock				10–14
1.	He*	OrK	184	398	46
1a.	He*/OrK	He*	5	419	1.2
1b.	He*	He*/OrK	98	174	56
2.	OrK	He*	0	539	0
2a.	He*/OrK	He*	3	416	0.7
2b.	He*	He*/OrK	48	276	17.4

whereas in the reciprocal cross no He* individuals appeared. The sexes were approximately equally represented in the He* F_1 individuals, from the He* female by Oregon K male cross, so that one cannot attribute the difference between the reciprocal crosses to a direct effect of recessive sex-linked factors. One must rather suppose that there is some influence of the mother, either by way of a maternal effect or possibly of a cytoplasmic nature. The series of crosses are, however, somewhat inconsistent with one another, in that many fewer He* flies were produced by the back-cross of a heterozygous He* male derived from an Oregon K mother on to an He* female (see line 2b) than had been produced when a pure wild type male had been used on an He* female (see line 1, F_1). The explanation of this was presumably to be found in the fact that there was considerable heterogeneity between the different cultures, which was taken to indicate that mass-mated He* stock was far from uniform in genotype.

An attempt was therefore made to purify the He* stock by carrying it for several generations in pair matings, amongst which those which gave the highest percentage of He* phenotypes were selected. After some generations of this procedure, a new series of matings were set up, both between the various phenotypes in the He* stock, and between the He* stock and an Oregon K wild

TABLE 4. *He*: second set of crosses to OrK*

	Parents' genotype		Parents' phenotype		Groups of pairs	Progeny		
	♀	♂	♀	♂		He*	Total	He*%
1.	He*	He*	He*	He*	10	264	387	68
					2	16	84	19
2.	He*	He*	+	He*	8	362	854	80
					1	11	85	13
3.	He*	He*	He*	+	12	429	658	63
4.	He*	He*	+	+	6	225	358	63
5.	He*	+	He*	+	—	90	592	15
5a.	He*	He*/+ ex 5	He*	He*	7	143	254	56
					1	3	29	10
5b.	He*	He*/+ ex 5	He*	+	7	91	247	37
					1	9	88	10
5c.	He*/+ ex 5	He*	He*	He*	—	7	653	1
5d.	He*/+ ex 5	He*	+	He*	—	3	358	1
6.	ex 5c	ex 5c	+	He*	3	117	187	63
					2	31	179	17
					4	14	225	6
7.	ex 5c	ex 5c	+	+	6	193	346	56
					6	9	517	2
8.	+	He*	+	He*	—	0	674	0
8a.	He*	+/He* ex 8	He*	+	—	298	610	49
8b.	+/He* ex 8	He*	+	He*	—	17	859	5
9.	ex 8b	ex 8b	+	He*	5	156	255	61
					4	8	311	2
10.	ex 8b	ex 8b	+	+	2	55	104	53
					9	14	300	3

type. As will be seen from table 4, lines 1–4, there is still some heterogeneity within the He* stock, but this had been very considerably reduced. Matings between individuals of this stock which had wild type phenotypes produced nearly the same number of He* offspring as did matings between individuals of He* phenotype, but a few pairs gave considerably fewer He* offspring than did the majority of matings. Thus it appeared that the stock had become more uniform for a genetic constitution which had produced He* phenotypes in a proportion of between 60% and 70%.

The results of crosses with wild type and back-crosses carried out at this stage are also given in table 4. It will be seen that once again the reciprocal crosses with the wild type give different results; those with a wild type mother giving no He* phenotypes in the F_1 (line 8), whereas in the opposite cross such phenotypes do appear (line 5), though in a considerably lower proportion than was found in the earlier series of crosses with the less purified strains. In the back-crosses of the F_1 males to He* females, the numbers of He* phenotypes appearing are now in reasonable agreement with one another (lines 5a, 5b, 8a). The most interesting result appears from the back-crosses of the F_1 females to He* males and the subsequent generations. The back-crosses themselves gave rise to very few He* phenotypes (lines 5c, 5d, 8b). However, when the female offspring of these back-crosses (wild type in phenotype) were themselves crossed to their brothers (either to wild type or He* in phenotype), the families seemed to fall into two distinct groups (lines 6, 7, 9,

TABLE 5. *Hatchability in unselected wild type and selected lines of Experiment I (generation c. 35), following 25 mins. ether vapour at 2½ hrs.*

Stock	Eggs treated	Hatched	Hatched %
Or.K.	400	270	67.5
Expt. I, down selected	500	408	81.6
Expt. I, up selected	500	418	83.6

10). One group gave about 55% to 60% of He* offspring, while another group gave a low percentage of the order of 2–5%. This result can be simply explained if we suppose that an important factor in the production of the He* phenotype in these stocks and crosses is a recessive gene which has no obvious effect on males but which causes females homozygous for it to produce eggs with a strong tendency to develop into He* phenotypes. This maternal effect gene must be supposed to co-operate with a polygenic tendency towards He,* dependent on minor genes scattered throughout the second and third chromosomes.

Other Effects of the Assimilation Procedure

The ether treatment necessary to produce the bithorax phenocopy causes a certain reduction in the percentage hatch of the eggs subjected to it. This was particularly the case at the beginning of the experiment. As shown in table 5, hatchability improved in both the upward and downward selected lines. Since in both cases the selection involved breeding from survivors, this improvement is not unexpected.

It is interesting to inquire whether selection for a high rate of phenocopy production by a certain environmental stimulus produces a specific sensitivity in the selected strain, or whether one finishes with a stock which is highly sensitive against any type of abnormal environment. This question has not been studied in detail, but the He* lines were given the hot shock treatment of the pupae which had previously been used to produce the crossveinless phenocopy, and the assimilated crossveinless strain, together with the wild Edinburgh stock from which it had been derived, were given the He treatment. The results are shown in table 6. It is clear that all the He lines had become rather more sensitive to the temperature shock to the pupae than was the wild type from which they originated. There is no evidence that the lines selected for low sensitivity to ether treatment are more resistant to the hot shock than the lines selected for high susceptibility: in fact the low line of Experiment I is the most sensitive of the group. The *cvl* line, selected for high susceptibility to hot shock, had reacted in the opposite way, and became considerably more resistant to the ether treatment than the Wild Edinburgh from which it originated. It seems then that the selections, which had certainly produced specific sensitivities or resistances to particular environmental stimuli, had not regularly resulted in corresponding changes in general developmental buffering.

TABLE 6. He *lines given* cvl *treatment (4 hrs. at 40° C.). Percentage of* cvl *phenocopies produced*

Stock	Time treatment applied after pupation: 19–21 hrs.	21–23 hrs.
He Expt. I		
Up selected	2.3	11.8
Down selected	9.3	24.3
He Expt. II		
Up selected	2.6	13.7
Down selected	6.9	12.9
Or.K.		8.1

Lines from *cvl* expt. given *He* treatment. Percentage of *He* phenocopies produced.

cvl assimilated line	6.0
Wild Edinburgh	21.1

Discussion

Selection for the ability to respond to ether treatment of the eggs by the production of bithorax-like phenocopies has on three at first sight separate occasions resulted in the appearance of stocks which exhibit this phenotype even in the absence of any special environmental stimulus. There were, firstly, the *He* stocks which arose in generation 8 of Experiment II, giving a *Bxl*-like adult; secondly, the similar stock (He 17) which arose in generation 29 of Experiment I; and thirdly, the *He** stock which appeared at the same time in Experiment I, but which gave a more *bx*-like phenotype, which was inherited in a different manner.

Such genetic assimilation of the abnormal phenotype might be due, in the first place, to the occurrence of a single new mutation which produces the character in question, and this mutation might be either spontaneous, or in some way caused by the treatment; or, in the second place, to the assembling together, by the selection pressure exerted, of already-present minor allelic differences tending in the appropriate direction; or finally, to a combination of these two mechanisms. The evidence strongly suggests that it is to the occurrence of new mutations that one must attribute the appearance of both the first group of *He* stocks and the second such stock, *He* 17. In both cases, the phenotype is dependent on a single gene, which produces its effect even in normal wild-type backgrounds, and there is no evidence that the accumulation of minor gene differences has played any significant role. Moreover, the genes are dominant in phenotypic expression, and cannot have been present in the initial population from which selection was begun.

Since the first lot of He stocks originated at quite a different time to *He* 17, and in a different selection line, the question arises whether we are dealing with two independent mutations; and if so, whether the occurrence of two apparently identical mutations in the populations involved would suggest that the treatment had caused them. As a matter of fact, since the selected stocks were basically wild-type in character, there is no absolute safeguard against the possibility that *He* 17 was a contaminant from the earlier *He* stocks which were of course present in the laboratory at the time it was found. However, concurrent experimental work has been little troubled by contamination, and there is no specific reason to suspect it in this case. If one adopts the alternative possibility, that two independent mutations to a *Bxl*-like allele have occurred, it is of interest to inquire whether the numbers of animals involved makes it all reasonable to postulate such a double event without supposing that the mutations were induced by the treatment. Unfortunately the records which were kept do not make it possible to give a completely accurate figure for the numbers. We are only concerned with the upward selected lines, since if such a mutation occurred in the downward lines, it would have been classified as a phenocopy and excluded from breeding. In Experiment II, rather over 17,000 flies had been treated and examined before the first *He's* were found; and to these must be added a fairly considerable, though unrecorded, number of untreated sibs which were examined (and amongst which the *He's* occurred). One can take it that at least some 20–25,000 flies had been inspected by the time the *He* mutation was found. Selection was then carried on for a further six generations in Experiment II, involving 14,000 flies examined. In Experiment I, some 87,000 flies had been inspected before *He* 17 was found in generation 29. If one makes allowance for examined untreated flies, one can conclude that a total of the order of 150,000 individuals were inspected in the whole upward selected lines of both experiments. The occurrence of two similar mutations amongst this number would give a mutation rate for the locus rather nearer 10^{-5} than 10^{-4}. Such a spon-

taneous mutation rate, though undoubtedly high, does not seem to be so excessive so that one is driven to postulate a direct inducing action by the environmental treatment.

In the other stock which shows assimilation, He*, the genetic changes are more far-reaching in their effects. The abnormal individuals produced by this stock are much nearer to the phenocopy, showing all grades of bithorax-like appearance up to an extreme type with a large second pair of wings and a considerable extra mesothorax. The assimilation has therefore been rather thorough. Its genetic basis certainly does not depend on the chance occurrence of only a single mutation producing this effect; the variation in the proportion of abnormal phenotypes in the various crosses clearly demonstrates that the character is affected by a polygenic system.

Since the phenocopy is induced by an environmental stimulus on the early egg, the effectiveness of which may well be supposed to depend on characteristics of the egg cytoplasm, it is perhaps not unexpected to find that an important part in the genetic constitution of the assimilated stock is played by a maternal effect. The full genetic analysis of this effect is still in progress and will be reported on later. The present data show that the genetic determinant or determinants of it are recessive. The relatively clean-cut segregation of the daughters of the back-cross of an F_1 female to an He* male into two classes (table 4, lines 9 and 10) suggests that the genetic basis of the character lies on one chromosome. It may indeed be a single gene, but it might be two or more fairly closely linked genes. If it is single, the question will arise whether it could have existed in the initial population before selection started. In crosses of He* females to wild-type males the maternal effect, operating on eggs which are heterozygous for the rest of the He* genotype, produces 15% of bithorax-like offspring. It is not yet known what effect, if any, the maternal effect genes can produce in a genotype which lacks any of the rest of the He* complex. If even in these circumstances it produces some bithorax-like phenotypes, one would have to conclude that the condition had not been present in the initial population but had arisen by mutation or recombination during the course of the selection. As has been pointed out, the number of individuals runs into six figures, so that an origin by mutation would not be anything out of the way.

Whatever the origin of the maternal effect gene or genes turns out to be, there can be no doubt that the selection has brought together and concentrated a considerable number of minor alleles tending in the bithorax direction. Many if not all of these were presumably present in the original population. The attempt to assimilate the bithorax character was originally undertaken with the idea that from the evolutionary point of view it was a very bizarre aberration and would provide a good test of the powers of the mechanism of assimilation. The fact that the assimilation has been successfully carried out in a number of generations, which although long in the laboratory are very short in the time scale of nature, suggests that the mechanism can in fact be an extremely powerful one. It seems likely that any modification produced by the environment could, if it were favourable to the animal, be genetically assimilated in a relatively short time.

It is instructive to compare the process of genetic assimilation as it has occurred in the He stocks with the "organic selection" of Baldwin (1902) and Lloyd Morgan (1900). They argued that if an animal subjected to any environmental stimulus is able to respond to it in an adaptive manner, the animal and the population of which it is a member will be able to continue existing in the region where the environmental stimulus operates, until such time as a chance mutation produces a phenotypic effect which mimics the adaptive response. Now this is what seems to have happened in the first two

sets of He assimilated stocks, in which a dominant allele of Bxl has appeared and produces a phenotype which has at least the lowest grade of the phenocopy appearance. The development of the full assimilation of the character in the He* stock can not, however, be accounted for in these terms. In the first place, one of the components of the genetic system, namely the maternal effect, is recessive. If we are to account for its appearance by some process other than selection for the capacity to respond to the environmental stimulus, we would have to take account not only of the low frequency with which such a mutation might be expected, but of the small probability that it would have emerged in homozygous state in these experiments, in which the inbreeding was very low. Moreover, there is no doubt that the maternal effect does increase the readiness with which the eggs respond to the ether treatment by developing bithorax phenocopies. It must surely be to this fact that its selection and concentration must be attributed, and the same applies to the other components of the He* genotype. We must be dealing with an "assimilation," produced by selection of capacity for response, rather than an "organic selection" of a chance mutation mimicing the response.

In a recent discussion by Underwood (1954) of the relevance of experiments on genetic assimilation to evolution as it occurs in Nature, some misunderstanding seems to have arisen as to what is actually being claimed for this process. It is quite true, as Underwood points out, that animals in Nature are rarely subjected to such extreme environmental stimuli as the temperature shocks used in the experiments on crossveinless phenocopies, while the ether treatment used here is even less "natural." Again it may be conceded that neither of these environmental treatments did, or could in the nature of the case, produce phenotypic changes which were adaptive. All this, however, does not in any way make it impossible that such experiments should reveal a general mechanism, relating genotypes to selective forces acting on environmentally-influenced phenotypes, which may be of general significance, and which may operate in an essentially similar manner when the environmental stimuli are milder in character and their phenotypic effects adaptive.

Another point raised by Underwood is the consideration that if a genotype can respond adaptively to the environment it normally meets, genetic assimilation of the adaptive phenotypic characters will be unnecessary. On *a priori* grounds, the argument may seem plausible; but the fact is that most adaptive features of animals are genetically fixed, and do not alter much when development takes place in somewhat unusual conditions. It is not entirely clear why this should be so; possibly it is because it is difficult to build up a genotype which develops with the right degree of adaptive modification to the whole range of environments it has to meet, and that it "pays better" to sacrifice something of the flexibility of the developmental system and to assimilate genetically the adaptation to the most usual environment.

Underwood suggests that "we should distinguish as *primary* (genetically) *fixed adaptations* those which we believe to have arisen as such, and as *secondary fixed adaptations* those which have arisen by fixation of a facultative adaptation." This implies that the environmental modification of development is quite irrelevant to the origin of the primary fixed adaptations; and the same view is implicit in Underwood's argument "if it be necessary in the end to invoke a change of genotype to establish the suitable embryonic inductor (of callosities in the ostrich) then it may surely be invoked in the first place." Now it is certainly very usual at the present time to discuss the evolution of new characters without ever referring to the effects of environment on development, or at best to bring this in at a late stage in the discussion, as a secondary factor in the way suggested by Under-

wood. But in my opinion such an approach is theoretically inadequate. All phenotypes are modified, to a greater or lesser extent, by the environment. All genotypes under natural conditions will be subject to selection pressures relating to the manner in which their development is modified by the environment. The phenotypic effect of any new gene mutation must therefore be to some extent influenced by the kind of developmental flexibility which has been built into the rest of the genotype by selection for its response to the environment. The effect of such specific developmental flexibilities may sometimes be rather slight (as it seems to have been in the first set of assimilated He stocks), or considerably greater, (as it was in respect of the maternal effect factor in the He* stock); but it must always be there. The distinction between primary and secondary fixed adaptations is therefore not a real one, as Underwood has drawn it.

There is not space here for a full discussion of all the implications of the views advanced above. It is sufficient to point out that the experiments on genetic assimilation have been designed to maximise the influence of selectively-determined developmental instabilities, since this was the aspect in the total situation to which attention was being particularly directed. The fact that they have succeeded in producing a change of macro-evolutionary magnitude in the short space of 30 generations of selection suggests that such instabilities may on occasion be of considerable importance.

Summary

1. Two replicate experiments were made, in which treatment by ether vapour was given to the eggs of Drosophila melanogaster 2½–3½ hours after laying, and selection practised either for the tendency to produce bithorax-like phenocopies ("upward selection"), or against it ("downward selection"). The two foundation stocks were both Oregon-K wild types, but had been bred separately for some considerable time.

2. Selection was effective in both directions, but its progress was not followed in detail owing to the death of many extreme phenocopies in the puparia.

3. In the 8th and 9th generations of the Up selected line of Experiment II, flies which showed a slight enlargement of the halteres appeared among the untreated individuals. These gave rise to a series of stocks, which were found to contain a dominant allele of *Bxl* with recessive lethal effect.

4. In the 29th generation of the Up selected line of Experiment I, similar flies were again found among untreated individuals. These gave rise to a stock (He 17), which contained a factor which was indistinguishable from that mentioned in para. 3. It might, indeed, have arisen through contamination by flies carrying that gene; but the numbers involved in the experiment (c. 150,000) make it conceivable that both occurrences of the gene were due to independent chance mutations.

5. In the 29th generation of the Up selected line of Experiment I there also occurred, among untreated individuals, some showing the metathorax partially converted into a mesothorax. These gave rise to a stock He*, in which the bithorax phenotype appears in high frequency and extreme grade. The genetic basis of the character is partly a recessive gene which causes females to produce eggs developing into bithorax phenotypes (a maternal effect), and partly a number of minor genes on both the second and third chromosomes.

6. Selection for sensitivity to one type of environmental stimulus does not necessarily produce susceptibility to stimuli of a different type.

7. The fact that such a bizarre phenotype as bithorax can be assimilated, with high grade expression, in less than 30 generations, suggests that the genetic assimilation mechanism is a very powerful

one, which could have far-reaching effects during evolution.

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SELECTION FOR SEXUAL ISOLATION WITHIN A SPECIES

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Two mechanisms have been advanced for the origin of reproductive isolation between species. Muller (1939), dealing in the main with barriers to crossing in the later stages of species divergence, such as hybrid inviability and infertility, suggests that these arise almost by chance as a product of change in the genetic background either by genetic drift or as adaptation to different biological situations. This would lead to accelerating divergences as the process continues, or, as Muller puts it, "ever more pronounced immiscibility as an inevitable consequence of non-mixing." Dobzhansky's suggestion (1937), which is perhaps complementary rather than antagonistic to Muller's, is that when sufficient divergence between two species has arisen so that the hybrids are less well adapted for any available habitat than either parental type, there will be selection for sexual isolation. That is to say—if mating can take place and if the resulting hybrids are inviable or infertile, then natural selection will operate to reduce the chance that mating will occur, either by reducing the chance of encounter or the chance of mating with members of the other species when they are encountered.

Some writers, in discussing the mechanism proposed by Dobzhansky have suggested that "natural selection will favour any mechanism which prevents the wastage of gametes involved in unsuccessful hybridisation." This seems to be unduly teleological. Natural selection will only tend to suppress crossbreeding if those individuals which hybridise will in consequence pass on fewer gametes in the form of pure-bred offspring. It would seem probable that this would be more often the case in females than in males. In *Drosophila melanogaster,* for instance, females seem reluctant to mate again for a period of two or three days after an effective mating. If the first mating has been heterogamic, this will reduce the number of purebred offspring that she will produce in her lifetime. Gestation in mammals will have a similar effect. But the male, who must on the average have the same number of effective matings in his life as the female, is usually capable of many more if willing females are available. It follows then that willingness to cross-breed, which may merely be a sign of greater general sexual activity, will not necessarily reduce the number of purebred progeny that a male will leave. If Dobzhansky's mechanism for the establishment of sexual isolation is correct, it follows that it should be in the main a matter of female preference. Merrell (1954) has recently presented evidence that it is the female which exercises discrimination in matings between *D. pseudobscura* and *D. persimilis.*

Koopman (1950) has shown that selection leads to an intensification of the sexual isolation between these two species. Using marked stocks of the two species, he selected continually for purebred flies—the progeny of parents that had mated homogamically. He showed that the proportion of hybrids emerging declined dramatically after a few generations of selection. More recently, Wallace (1950) and King (private communication) attempted to demonstrate the production of sexual isolation by selection within a species. They used two stocks of *D. melanogaster,* from widely separated localities, which had each been marked by a different recessive gene. After 12 generations, when the experiment was first reported, little change in the proportion of wild-type flies emerg-

ing had been observed, but in subsequent generations the proportion declined significantly, showing that sexual isolation had been to some extent established. This was confirmed by observation of individual matings.

Our own experiment on very similar lines was started before we were aware of Wallace's work, and as our work was slightly different in conception, we decided to proceed with it. In Wallace's experiment, the mutants were used solely as markers, the stocks because of their origin presumably differing in many genes. As it happens, we had used in our work stocks marked with the autosomal recessive genes, ebony and vestigial, which has been extracted from a population in which the two had been segregating for many generations. The original stocks making up this population were actually those used by Rendel (1951) in his work on the effect of light on the mating of these mutants. Our two foundation stocks, both of which contained a considerable amount of genetic variability, were thus probably genetically very similar except for the marker genes. These genes were chosen because of the ease of scoring but they do react differently to light and, as Rendel has shown, ebony males mate more frequently in the dark than in the light.

In the first experiment of this type that we carried out, there appeared in the seventh generation some flies that were both ebony and vestigial, indicating that in previous generations either a non-virgin female or else a wild-type heterozygote had been used as a parent with the result that each mutant stock was contaminated with the other gene. Theoretical consideration of the effect of this showed that the proportion of double recessive flies should increase by a factor of four each generation until they reached a level of 11% of all flies emerging. At that point, the proportion of flies in each mutant stock that were heterozygous for the other gene would be $\frac{2}{3}$. There would then be a continual interchange of genes between the two stocks. In addition, one-third of the apparently pure mutants used as parents would be derived from heterogamic matings, thereby reducing the selection for sexual isolation. We therefore discarded the line and started afresh with stringent precautions against non-virginity, parents being collected over a 7 hour period. In the two experiments presented here in detail, no double recessive flies were ever observed.

Description of Experiments

Box Experiment

Two mutant strains of *D. melanogaster* homozygous for the genes ebony and vestigial respectively were used. They had been extracted from the same population, after segregation for many generations. At the start, 54 males and 54 virgin females from each of the stocks were put together into a breeding box (size 18″ × 18″ × 7″) which contained 10 unstoppered ¼ pint bottles of maize meal–molasses–yeast–agar medium. Flies were etherised for counting, but were not put into the box until three hours after complete recovery from anaesthesia. The box was then placed in a constant temperature room at 25° C. All phases of the experiment were done at this temperature. The box was always put in the same part of the room, where, due to the direction of the light, two sides of the box near the edge were in slight shadow. The ebony flies, immediately the box was positioned, migrated towards the light source, that is, towards the shaded edge. After some time, the majority of them moved more freely about the cage.

After six days of mating, the ten food bottles were removed, cleared of any flies which remained inside, and stoppered. The parents were discarded. The count of the next generation was started five days afterwards, i.e., on the eleventh day after the parents were put into the box. Three types of flies emerged; hybrids from heterogamic matings, and the two mutants ebony and vestigial from

homogamic fertilizations. For 3½ days every fly which emerged was counted. The culture bottles were completely cleared at 10 A.M. Flies which emerged by 5 P.M. on the same day were segregated and mutants were kept in separate vials to be used as parents for the next generation when 1 to 4 days old. When insufficient virgins were obtained, those collected were bred with their own kind, and the experiment carried on from their progeny. In the box experiment, this was done three times in 38 generations.

In order to ensure that any changes in external conditions had not affected the course of the experiment, controls were done on the box experiment in the later generations. Parent virgin flies were obtained from the original stocks and put into a box of identical proportions to the experimental one. The control box and the experimental one received exactly the same treatment throughout. This was done seven times between the 25th and 35th generations.

Jar Experiment

An experiment on similar lines was run in conjunction with the cage one. A 2 lb. glass jar containing approximately 1″ of food was used as the breeding chamber. The number of parent flies employed in this case was between 20 and 30 of each sex of the mutants. Again, it was sometimes necessary to mate the virgins with their own kind to produce sufficient numbers for the next preferential mating. This was done three times in 33 generations. From generations 1 to 12 the parent flies were still under ether when put into the jar, as it was thought that they might otherwise escape. This was found, however, to be unsatisfactory. So from the 13th generation onwards the parents were introduced into the jar three hours after recovery from the ether. The jar was put into the same constant temperature room and at the same time as the box. Thereafter, all operations, such as clearing parents from the jar, counting and segregating flies of each generation, etc., were carried out at the same time and in an exactly similar manner to the box experiment.

Because of the small capacity of the jar compared with that of the box and the fact that there was little or no variation in the light within the jar, it was assumed that any tendency towards an eco-

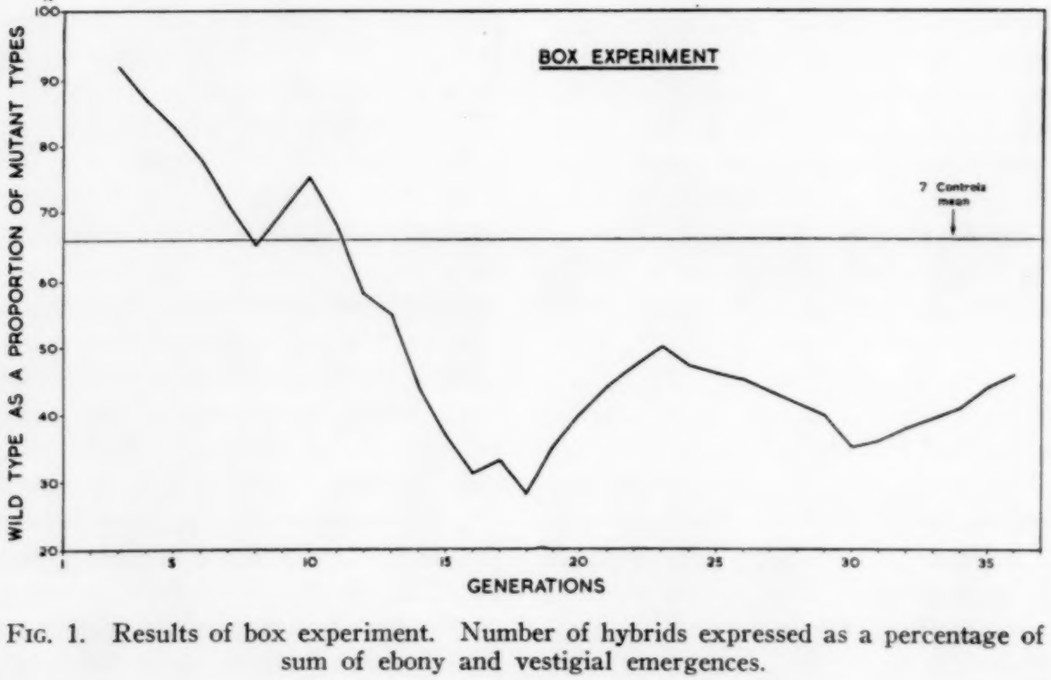

FIG. 1. Results of box experiment. Number of hybrids expressed as a percentage of sum of ebony and vestigial emergences.

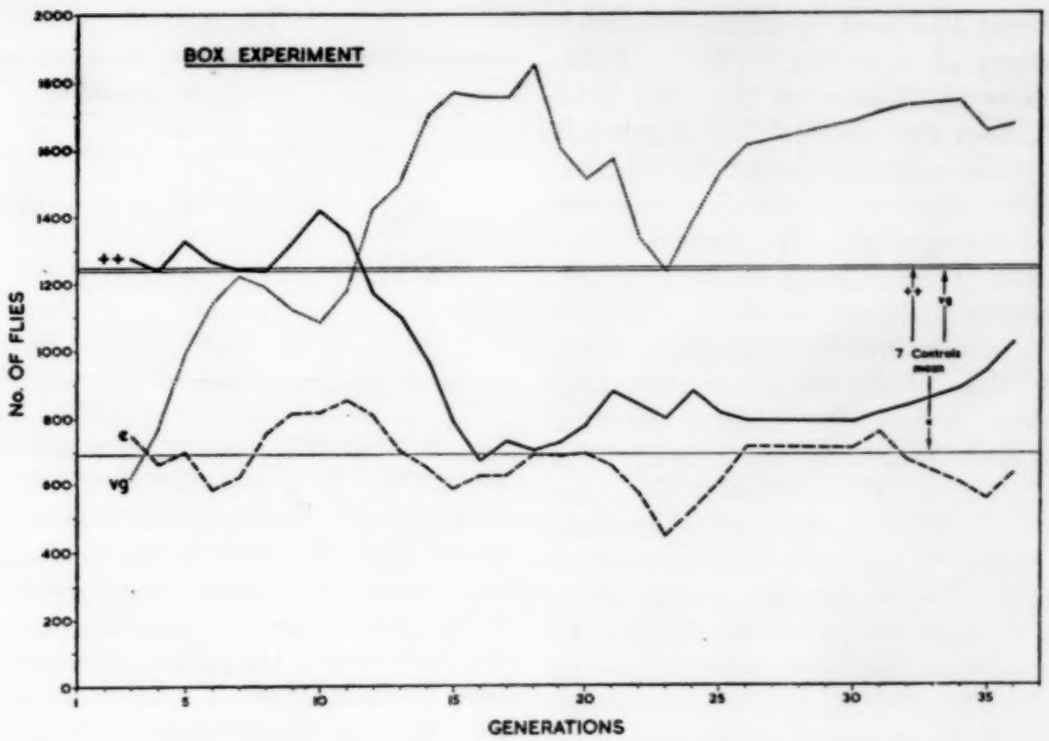

FIG. 2. Results of box experiment. Numbers of 3 types of flies emerging shown separately.

logical isolation between the ebony and vestigial flies would be eliminated.

RESULTS

One of the first impressions at the start of the experiments was of the great fluctuation in results from generation to generation. The jar experiment was in fact started to try to remove this by having all flies developing in one food mass. Our criterion of isolation has been the ratio of wild-type flies, produced by heterogamic matings, to the total number of mutants produced by homogamic matings. The standard deviation of this ratio due to chance fluctuation, determined from the mean square difference between successive generations, was 0.16 for the box experiment and 0.24 for the jar. This fluctuation is equal to that produced by random sampling of 160 units and 70 units respectively from a population made up of two types of objects with equal frequency. The total count was actually of the order of 2,000 flies in both cases. But the number of female parents was 108 and 50 in the box and jar respectively. The observed fluctuations suggest that the effective units are the initial inseminations of the individual females. In this respect, it is of interest that of the individual platings of females taken from the box after six days of mating, 660 gave offspring all of the same type and only 75 had mixed offspring. However, whatever the reason, it is still true that too many flies were counted each generation and a sample of a quarter of the size that we took would have been quite adequate.

Box Experiment

The results of the box experiment are set out graphically in figures 1 and 2. The graphs are moving averages over 5 generations to smooth out fluctuations. In figure 1, the number of hybrids is expressed as a percentage of the sum of the ebony and vestigial emergences. Figure 2 shows separately the numbers of the three types of flies emerging. From the first to the eighteenth generation a more or less steady decline in the percentage of hybrids is noted. The lowest percentage of hybrids in any individual gen-

eration was 10.3% at the eighteenth, with emergences of ++ 246, e 736, vg 1640. Only once afterwards, at the 23rd generation, does the vestigial line graph fall as low as the control mean for this mutant. Thereafter the values remain high for vestigial emergences. The hybrid figure drops, and is lowest between the 16th and 18th generations, only rising a little and slowly towards the end of the experiment. During the whole 38 generations the emergence values for ebony alternate slightly above and below the figure for the control mean. This suggests that the sexual isolation, after the 18th generation, is due mainly to the increase in the number of homogamic matings of the vestigial flies.

The average values for the seven control generations are also shown in figures 1 and 2. The proportion of wild-type flies to mutants averages 0.66, compared to the proportion in the selected population at the same period of 0.38. The figures for the individual mutants show that the change is due to a decrease of wild-type flies and an increase of vestigial.

It has been shown by Rendel (1951) that ebony reacts to light intensity in its mating behaviour. It seemed possible that the sexual isolation was due to an accentuation of this response. Towards the end of the experiment, therefore, duplicates of the selection box were made up from parents from the selected stock but were kept instead in complete darkness. The ratio of wildtype to mutant offspring was 0.48 compared to 0.46 for the three contemporary generations in the light. It seems therefore that the demonstrated sexual isolation is not concerned with phototropic response. However, there were many more ebony flies in the dark boxes—in fact the average of the three tests (1102 flies) had only once been exceeded by a single generation in the light, and the average in the last few generations of the latter was about 650. There was correspondingly a shortage of vg flies, but the proportional effect was not so great. This agrees with Rendel's observation that ebony males show greater sexual activity in the dark.

TABLE 1

Controls ♀	Inseminated by e	Inseminated by vg
e	71	69
vg	41	63
Selected stocks		
e	151	108
vg	77	142

Between the 20th and 30th generations, the females were placed in individual vials after they had been removed from the box, and their progeny were examined on emergence. This was done with 6 generations of the selected stocks and with three of the controls. The results in terms of effective matings are given in table 1.

There is a slight tendency to homo-

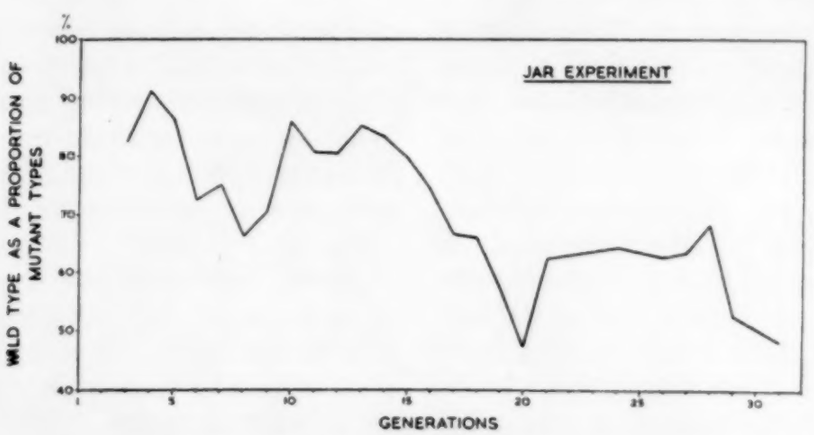

FIG. 3. Results of jar experiment. Compare figure 1.

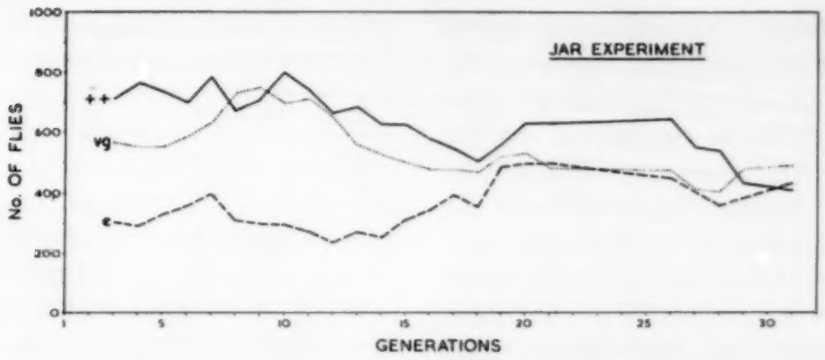

FIG. 4. Results of jar experiment. Compare figure 2.

gamic mating in the controls but the heterogeneity χ^2 is only 2.15. In the selected stocks the tendency is much more marked and the χ^2 value is 27.14. This is confirmatory evidence that some degree of sexual isolation has been obtained in the selected population.

It might have happened that this type of selection, picking out always the mutant flies, would have affected the segregation ratio by selecting those genes favouring the survival from egg to adult of the mutant types. However, a check based on several thousand flies at the end of the experiment showed no differences between control and selected stock in the segregation ratio for either mutant.

Jar Experiment

The results for the jar experiment are given in figures 3 and 4. In the jar, the light intensity was much more uniform than in the box and in addition the flies were more confined—the volume of the jar being of the order of $\frac{1}{50}$ of that of the box. Here again there is a decline in the proportion of wild-type flies as the experiment proceeds, although the proportion at the end is a trifle higher than in the box experiment. However, the ratio of wild-type to mutant flies has declined from 80% to between 50% and 60%. As was noted above, there was a change in method in the middle of this experiment. Up to generation 12, flies were put into the jar etherized but afterwards they were put in an active state. This change does not appear to have affected the sexual isolation. In the five generations before the change, the average ratio of wild-type to mutants was 0.86 and in the five after the change it averaged 0.80. It may however have affected the separate types. The numbers of vg and wild-type flies decline by about one-third as a result of the change, whereas the ebony count is unchanged. The subsequent change in the wild-type/mutant ratio appears in this case to be due to an increase in the number of ebony flies. Control experiments were not carried out on the jar population, as the latter was subsidiary to the main experiment in the box.

Sexual Preferences in Inbred Lines and Closed Populations

It is convenient to present here a small amount of data on sexual preferences between lines and populations chosen at random, with a bearing on the "chance" occurrence of sexual isolation. These experiments were carried out by the usual "male choice" method in which males are given equal numbers of two types of female, one of which is recognisable—in our case by a spot of silver paint. The females are then examined for the presence of sperm in the seminal tract. In the first case, two wild-type inbred lines of completely different origin were used, given in our stock list the symbols W20 and K7. The results are given in table 2, which shows the proportion of females inseminated. In all cases, the ♂♂ were equal in number to the ♀♀ of each of the separate lines.

A similar experiment was then done

using lines which, although of common origin, had been selected in different directions without inbreeding for 20 generations for number of chaetae on the 4th and 5th abdominal sternites. There was no overlap in chaetae count between the high and low lines used, so that this character could be used for identifications. The results are given in table 3, for matings between one high line, H1 and two low lines, L4 and L5.

In these rather meagre results, there is little suggestion of sexual isolation having developed by chance in either the inbred experiment or in that with the selected lines. In the latter, it is of interest that the selection for the quantitative character has caused a differentiation in mating ability. The H1 ♂♂ are poorer than those from the two low lines but on the other hand the high ♀♀ seem to be better. But this seems to be a general change in sexual drive, not specifically adapted to the other sex of the same line. Wallace (1955 in press, and personal communication) has also tested whether the mere isolation of two populations is sufficient to cause sexual isolation to arise between them. His populations had been separated for 80–100 generations, and differed in certain morphological characters (primarily abdominal pigmentation). In an extensive series of tests, no tendency towards preferentially mating could be detected nor does there seem to have been any evidence of differences in the intensity of general sexual drive.

TABLE 2

♀s marked	♂	W20 ♀	K7 ♀	Duration of mating
W20	W20	22/23	11/25	40 mins.
	K7	17/23	9/25	40 mins.
W20	W20	17/19	16/20	70 mins.
	K7	4/20	1/19	70 mins.
K7	W20	9/10	5/10	30 mins.
	K7	8/14	9/14	60 mins.
K7	W20	10/20	2/19	30 mins.
	K7	10/20	5/20	45 mins.

TABLE 3

♂	H1 ♀	L4 ♀	Duration of mating
H1	3/10	1/9	30 mins.
L4	9/14	3/14	30 mins.
	H1 ♀	L5 ♀	
H1	11/20	5/19	60 mins.
L5	20/24	17/25	60 mins.

DISCUSSION

Our results may be summarised in the statement that some sexual isolation developed when we selected for a tendency towards homogamic matings, but that none was found to have arisen by chance in a few lines which had been selected for abdominal chaeta number or inbred. Laboratory experiments on evolutionary mechanisms can, of course, only be indicative and not demonstrative—they can show what might happen in wild populations, rather than what has happened. As far as they go, our experiments lend support to the mechanism suggested by Dobzhansky rather than that discussed by Muller. But when attempting to apply these results to occurrences in nature, one must bear in mind the ways in which artificial populations may fail to imitate conditions in the wild.

It is perhaps misleading to put Muller's hypothesis of the chance origin of sexual isolation in antithesis to that of Dobzhansky, which attributes it to the action of selection. In all probability, both mechanisms have operated in the wild in different cases. Some evidence supporting Dobzhansky's hypothesis comes from Dobzhansky and Koller (1938) who found, in an analysis of crosses between *Drosophila pseudoobscura* and *D. miranda*, that the isolation was greatest between races close to each other in their range. King (1947) has similar evidence from the *guarani* group. However, even between races of the two species widely

separated in origin, the isolation was considerable. One has the suspicion that sexual isolation is common between species which have never had the opportunity to crossbreed, though the evidence is rarely conclusive since seldom if ever do we know the full evolutionary history of the populations.

It is perhaps not surprising that differences in sexual behaviour arose in the experiments involving selection, but were not found in the comparison of populations which has originated independently. If they had occurred in the latter, they could only have appeared by chance, or as a correlated response. It might be expected that random changes in sexual behavior would be slow, even though they arose as a secondary response to an adaptive change in the population. Mating involves the cooperation of the two sexes and it seems unlikely that a genetic shift in the population causing a change of sexual behaviour in the female, perhaps by a modification of the pattern by which a male recognises an animal of his own species, would also change male behaviour in a compensating manner (although an exception to this might be in habitat preference). . An individual with aberrant sexual behaviour is not likely to leave many progeny. A population gradually changing its genetic situation could only change its pattern of mating by the selection of males capable of responding to the altered female behaviour. This must constitute a brake on the change of mating behaviour either by chance changes in the genetic situation or even as a correlated response to an adaptive change. This will be particularly true of inbred lines in which selection between potential mates is small or non-existent. Reproductive behaviour, excepting perhaps choice of habitat, would therefore be more stable than other physiological systems to genetic changes.

Both the hypotheses that we have discussed demand the development of a previous geographic isolation before sexual isolation can be established. In this sense, the selection hypothesis is perhaps clumsy, since in the formation of a new species showing sexual isolation with the parent species it demands first a geographic isolation and then an overlapping of the species range so that members of the two species can be selected for refusal to crossbreed. It seems to us that sexual isolation instead of being a consequence of geographic isolation, may be a contributory factor in its establishment. The spread of a population into new territory will often involve the occurrence of genotypes with new hereditary habitat preferences. The existence of such preferences amongst Drosophila stocks has recently been shown by Waddington, Woolf and Perry (1954). In organisms such as birds, in which rather sudden changes in geographical or ecological range are well-known, learning may play a part, but this may also have an important genetic component. In the genetic constitution of a sub-population which has broken out of the original species boundaries and is spreading into new territory, one must expect to find that a number of adjustments are occurring simultaneously. There is most likely to be, in the first place, an evolution of a new system of habitat-preferences and/or of general activity; in the second, the adaptive characters and general fitness of the migrating group will be attuned to the new circumstances which it has to meet. Both these necessary modifications of the gene pool will be made more easily if the genetic constitution of the sub-population is prevented from continual intermingling with that of the original stay-at-home group. Thus any tendency for preferential mating within the migrating group, and sexual isolation between it and the main population, will acquire selective value. It seems rather probable that a species may be able to spread into new territory even if no sexual isolation develops between the main population and the migrating one; but if the increase in species-range demands considerable adjustment of the genotype to fit the new

environment, the evolution of some degree of mating-barrier will undoubtedly be of considerable advantage. Our experiments show that the necessary genetic variability is likely to be present in a population; and the fact that the change of environment involves alterations to the behaviour pattern of the migrating animals makes it more likely that their preferences for sexual partners as well as for habitats, will exhibit evolutionary flexibility. Thus species-spread and sexual isolation will tend to act synergistically.

Summary

1. Partial sexual isolation (between two stocks of *D. melanogaster* differing only in marker genes) has been established by selection of the offspring of flies mating with their own type. This has been demonstrated by a reduction in the number of cross-bred offspring found and also by examination of the progeny of individual females.

2. In a small series of tests, no tendencies towards preferential mating were found to have arisen by chance in a sample of lines which had been inbred or selected for number of abdominal chaetae, although there were differences in intensity of sexual drive.

3. Changes in reproductive behaviour brought about by selection are more likely to affect female than male behaviour. Willingness to cross breed, in a male, will not materially reduce the number of his pure-bred offspring but in a female it usually will.

4. It is argued that selection pressure against cross-breeding of two partially separated populations, although probably effective when it occurs, is not likely to be the only mechanism by which sexual isolation between taxonomic groups develops in nature. It is suggested that an important part in the origin of such isolation may be played by the factors (e.g. changes in hereditarily controlled behaviour patterns) which bring about the spread of an initial panmictic population into new geographical or ecological situations.

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POLLEN DIMORPHISM IN THE RUBIACEAE

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Introduction

The striking dimorphism of pollen and stigmata found in the majority of the species of the tribe Staticeae in the Plumbaginaceae was discovered by McCleod (1887). This dimorphism and derived monomorphic conditions have been extensively investigated with results that have been found extremely helpful in the elucidation of genetical, genecological, phytogeographical and evolutionary problems in the family, particularly in the genera *Armeria* and *Limonium* (Baker, 1948*a*, *b*, 1950, 1952, 1953*a*, *b*, 1954*a*, *b*, *c*).

The extreme usefulness of this pollen- and stigma-dimorphism in investigations within the Plumbaginaceae suggests that similar phenomena should be looked for elsewhere. As there are at least 15 families in which heterostyly has been recognised (Bateman, 1954), there are at least this number of potential sites for pollen dimorphism, for it is in association with dipolar incompatibility systems that this kind of dimorphism is most likely to arise (Baker, 1954).

As a preliminary to a comprehensive examination of the pollen of these families a search has been made through the existing literature. This has revealed very little that is hopeful. Differences in the sizes of grains from long-styled and short-styled plants are usual, of course. Darwin (1877, p. 135) noted that soaked pollen grains from short-styled flowers of *Cinchona micrantha* showed sharper angles than those from long-styled flowers. On the other hand he found that slight differences in shape between the pollen-grains of long- and short-styled forms of some *Primula* species disappeared when the grains were wetted. However, when grains are prepared for examination by acetylation these differences persist (cf. Erdtman, 1952). More recently, Johnston (1953) has shown that there is a significant difference in shape between the grains of the corresponding forms of certain species of *Lithospermum*. A difference of another kind was found by Fritz Müller who reported that the pollen of the short-styled flowers of a species of *Villarsia* was bluish in colour while the pollen from long-styled plants was yellow (cf. Darwin, 1877, p. 116).

The impressive differences between the pollen forms in the Plumbaginaceae, however, lie in the sculpturing of the exine of the grains. In *Linum*, there is sometimes a small difference between the pollen of long- and short-styled plants in the verrucoid excrescences by which the grains are ornamented (see Erdtman, 1952) but the difference is slight and, it would seem, not always distinguishable.

Only one report of pollen dimorphism comparable in clarity with that in the Plumbaginaceae has been encountered and this, strangely enough, appears to have been missed by those who have surveyed the literature during the compilation of recent works on pollen morphology. It is the account by Fritz Müller (1869) of extreme pollen dimorphism in a species of *Faramea* (Rubiaceae) from Itajahy in the Brazilian State of Santa Catarina. That this should have been overlooked is especially surprising for Müller's account was re-presented (with illustrations) by Darwin (1877, p. 128–131) in his well-known book "The Different Forms of Flowers on Plants of the Same Species" (fig. 1). In addition, it was abstracted by Fritz's brother Hermann (H. Müller, 1883) in "The Fertilization of Flowers."

According to Fritz Müller, the pollen grains of the short-styled form of the species in question are about $\frac{1}{12}$th millimetre in diameter while those of the long-styled form measure only about $\frac{1}{18}$th millimetre. The most striking difference, however, is that the grains of the short-styled form bear an ornamentation of short spines (as in the Malvaceae) while those of the long-styled form are absolutely smooth.

As this paper was written at a time when Darwinism was making its first, strong impression on the minds of naturalists (and as Müller was a close correspondent of Darwin's) it is not surprising that Müller immediately thought of an adaptive advantage in this morphological difference. He remarked upon the greater difficulty of removing the spiny pollen from the exserted anthers of the short-styled form and concluded that this prevented excessive loss of pollen through wind-action although the grains would readily adhere to the hairy abdomen of a visiting insect which might brush against them. Such protection would not be necessary for the pollen produced by the anthers of the long-styled form which are enveloped by the corolla-tube and are brought into close contact with the mouth-parts of visiting nectar-seekers.

Another interesting difference between the two flower-forms is to be seen in the manner in which the anthers are borne in the mature flowers. Thus, in the buds of both forms the anthers are introse and they remain like this in the inserted stamens of the fully developed long-styled flower. As the short-styled flowers develop, however, there is a tendency for the filaments to rotate through 180° as they elongate and, at maturity, most of the anthers are more or less extrorse. To Müller, it was apparent that this is an adaptive process (for placing the pollen in the most advantageous position) which is in the course of perfection by natural selection.

There was also a difference in the form of the stigmata, those of the long styles being short and broad while those of the short styles are long, narrow and sometimes much curled.

This, then, was the situation described by Müller in his paper written in April 1869. It was without precedent and at least one reader, Charles Darwin, found it hard to credit. It is clear that Müller wrote of his discovery (possibly with drawings) in a letter to Darwin prior to composing his paper for the Botanische Zeitung for we know from what remains of Darwin's correspondence that, on March 14th. 1869, the latter replied to Müller

> "Your case of *Faramea* astonished me. Are you sure there is no mistake? The difference in size of flower and wonderful difference in size and structure of pollen grains naturally make me rather sceptical. I never fail to admire and to be surprised at the number of points to which you attend" (F. Darwin & Seward, 1903, pp. 354–5).

Although there is no way of discovering the exact nature of Müller's answer to this letter, it is apparent that Darwin found it convincing for, eight years later he published the story in his book with only this remark

> "Until within the recent period, if any one had been shown two plants which differed in a uniform manner in the length of their stamens and pistils,—in the form of their stigmas,—in the manner of dehiscence and slightly in the size of their anthers,—and to an extraordinary degree in the diameter and structure of their pollen-grains, he would have declared it impossible that the two could have belonged to one and the same species" (Darwin, 1877, p. 131).

It is not unlikely that one of Müller's letters contained drawings for there is otherwise no clue to the origin of the illustrations on page 129 of Darwin's book (fig. 1). The caption to these is ambiguous and gives no clue as to whether they were drawn by Darwin, by Müller or by some third person. They were not taken from Müller's paper in Botanische Zeitung for this contains no illustration at all.

Fig. 9.

Short-styled form. Long-styled form.
Outlines of flowers from dried specimens. Pollen-grains, magnified 180 times, by Fritz Müller.

FARAMEA [sp. ?].

FIG. 1. Photocopy of figure 9 (with caption) on p. 129 of "The Different Forms of Flowers on Plants of the Same Species" by Charles Darwin (1877). Reproduced by kind permission of the publishers Messrs. John Murray.

It became obvious that there was need for a thorough check on this interesting but neglected account. *A priori* there were three possible explanations:

1) The long-styled and short-styled flowers came from different species, in which case the differences in pollen-morphology would not be remarkable. Supporting this explanation are:

(a) The difference in sizes of the flowers and the differences in the calyces as shown in the drawings.
(b) The difference in the directions of dehiscence of the anthers.
(c) The difficulty of species-determination (or even of satisfactory reference to genera) in the abundant Rubiaceae of Brazil.

2) A single species is represented but it is dioecious rather than heterostylous. Skottsberg (1944) has shown that a number of Hawaiian members of this family formerly considered to be heterostylous are functionally dioecious, the "short styles" indicating the partial abortion of the gynaecium while the "short" filaments in long-styled flowers bear anthers devoid of functional pollen. In support of this explanation for the present case is the smaller size of the long-styled, apparently female flower. This is a general tendency in taxa in which dioecism (or gynodioecism) is a relatively recent evolutionary development (Baker, 1948*c*). In *"Faramea,"* then, the smaller grains in the long-styled flowers would be "bad" (i.e., non-functional) pollen and their lack of ornamentation might well be correlated with their arrested development.

3) A heterostyled species with two very different pollen forms does exist as Müller maintained. Even if this be, true, however, there is no certainty that the species represented really belongs to the genus *Faramea*.

In attacking this problem there were two lines of approach. Firstly, the flower morphology and pollen of authenticated specimens of various species of *Faramea*

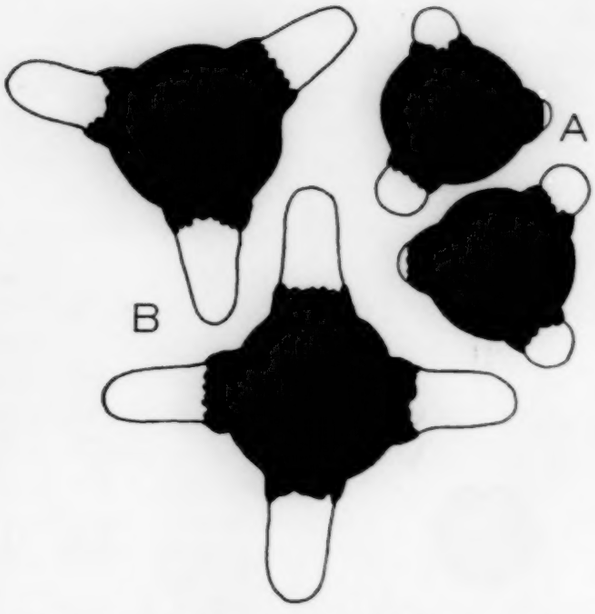

FIG. 2A. Triporate and tetraporate pollen grains of *Faramea occidentalis* showing prominent aperture-membranes. (×.) 2B. Pollen grains of *Faramea salicifolia* Presl. with bulbous aperture-membranes. (×.)

could be examined and, secondly, herbarium specimens of Müller's original plants could be sought.

RE-INVESTIGATION OF *Faramea*

Pollen Morphology

The morphology of the pollen of this genus has been given considerable attention by Erdtman (1951, 1952) in view of its interesting resemblance to certain Cretaceous sporomorphs from Sweden (Ross, 1949; Fries & Ross, 1950). At first sight, however, there is little resemblance between the triporate grains found in at least 20 species and figured for some by Erdtman (1951) and the non-aperturate grains illustrated in Darwin's book. Consequently, a personal investigation was made of the pollen of two species of *Faramea,* the grains being obtained from specimens of authenticated material in the Herbarium of the Royal Botanic Gardens, Kew. For permission to do this the author is greatly indebted to the authorities concerned.

Faramea occidentalis (Jacq.) A. Rich. and *F. salicifolia* Presl were chosen, the former (a West Indian species) because it was the most abundantly represented and the second because it is the most frequent species of this genus in the neighbourhood of Itajahy in the State of Santa Catarina, Brazil, where Müller collected his material.

Some of the results of this investigation have been reported elsewhere (Baker, 1955) for, although they proved conclusively that Müller's flowers could not have come from the genus *Faramea,* they revealed intrinsic features of considerable palynological significance. It appears that the porate pollen of *Faramea* is characterised by extremely prominent aperture membranes (figs. 2A, B) and that these membranes, whose shapes may provide characters of value in distinguishing the pollen of different species within the genus, are easily destroyed by the techniques often employed in the preparation of grains for microscopical examination. Within a species the only variation is in the proportions of triporate and tetraporate grains.

Flower Morphology

The flowers illustrated in Darwin's book are pentamerous (with inconspicuous calyces and long corolla-tubes bearing narrow petal-lobes). They are also heterostyled (or dioecious). In one form the stamens are strongly exserted (with the anthers more or less extrorse) while, in the other, the short bifid stigma is correspondingly exserted.

The two species of *Faramea* which were closely examined (and the remainder which were looked at) failed to correspond with this flower morphology. The genus *Faramea* is characteristically *tetramerous* from the calyx to the androecium and there was no indication of heterostyly. Because of Fritz Müller's publication of his observations in Brazil and their subsequent summary by Darwin (1877) and Hermann Müller (1883), *Faramea* is sometimes included in lists of heterostyled genera (e.g., Knuth, 1909). Most sur-

prisingly, Schumann (1891), who monographed the family, appears to have taken this on trust as well. Nevertheless, botanists who have investigated this genus in the field and who are well aware of heterostyly in other genera give no indication that it ever occurs here (e.g., Bremekamp, 1934; Moore, 1936). In the present examination of herbarium material of *Faramea occidentalis* and *F. salicifolia* no exserted anthers were ever seen, they were constantly found included in the corolla-tube. The dissection of flower-buds showed that prior to anthesis the style does not reach up to the level of the anthers but that it subsequently elongates and carries the stigmata to the mouth of the corolla-tube. However, even the style does not become exserted to the extent figured in Darwin's book.

In these circumstances it is obvious that neither the long-styled nor the short-styled flowers in Müller's material could have belonged to the genus *Faramea*. Consequently it was both disconcerting and stimulating to read in his paper (F. Müller, 1869, p. 606) that he had sent specimens to Kew where they had been referred to this genus.

Herbarium Material at Kew

The first clear fact to emerge from the search for Müller's specimens was that he had sent material of *"Faramea"* at approximately the right date. Thus, in the appropriate volume of the Accessions List at Kew it is recorded that material was received from Fritz Müller and that, along with some other Rubiaceous specimens, nos. 119 and 125 were "Faramea sp."

One sheet was soon found in the folder containing *Faramea salicifolia*. Its label reads "No. 125 Faramea sp? com. Fritz Mueller 6/68. St. Catharina, Brazil." It agrees fully with the other sheets of *F. salicifolia* both in the gross morphology of the shoots and in the structure of the

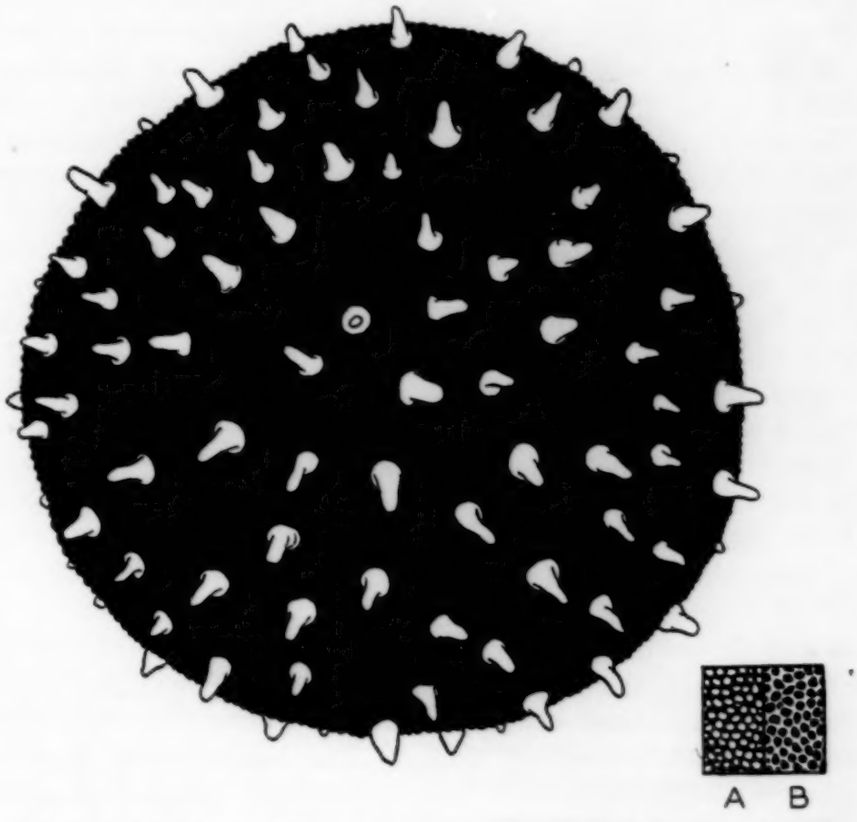

Fig. 3. Spiniferous pollen grain from short-styled *Rudgea jasminoides* (Cham.) A. Rich. (× 900.) Inset appearance of outer surface of grain between spines at A) highest focus, B) lowest focus.

pollen grains. However, it appears to have no direct bearing upon the present problem.

Consequently, attention was focussed upon Accession no. 119 which was probably collected at much the same time. It was not to be found in any folder containing a species of *Faramea* and the conclusion was inescapable that, despite its original determination, a subsequent decision had been made that it belonged to some other genus. This was to be expected in view of the discrepancy between the illustrations in Darwin's book and the known floral characters of *Faramea*.

A search through the genera and species of the Rubiaceae would have been a tremendous task except that there were some points for guidance. A pentamerous, heterostyled genus with a representative species in southern Brazil was indicated. Three such genera are *Psychotria*, *Rudgea* and *Palicourea*. Despite the encouraging feature of non-aperturate pollen in *Palicourea* (exceptional in the family according to Erdtman, 1952), it was decided that the characteristic tuck in the corolla-tube in this genus was unlikely to have been missed by the illustrator of the flowers for Darwin's book and, as a consequence, *Psychotria* and *Rudgea* were selected for attention.

After an extended search the desired sheet was found in the folder of *Rudgea jasminoides* (Cham.) Muell.-Arg. It bears the label "No. 119. *Faramea* sp? Com. Fritz Mueller 6/68. St. Catharina, Brazil." Thus it was collected at a quite appropriate date to be a voucher for material used in the preparation of a paper written in April 1869. The specimen in question was determined as *Rudgea jasminoides* by P. C. Standley and upon this authority was transferred from a *Faramea* cover to the present *Rudgea* cover.

A flower-bud was removed from this specimen and dissected, revealing that the flowers were long-styled. Pollen from this bud consisted of spherical, non-aperturate grains with a very slightly granulate surface (fig. 4) agreeing closely

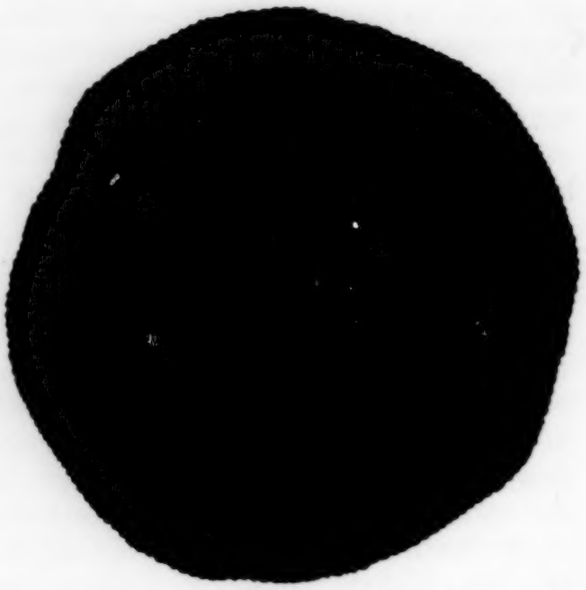

FIG. 4. Smooth pollen grain from long-styled *Rudgea jasminoides*. (× 900.)

with those figured in Darwin's book and described by Müller.

It appears that Müller did not send any specimens of the short-styled form but there are collections by other workers whose identifications have also been confirmed by specialists (including P. C. Standley). One of these, collected in 1861–2 by J. Weir, is short-styled. A flower-bud from this specimen revealed the larger, spiny non-aperturate grains that would be expected if *Rudgea jasminoides* were truly Müller's mysterious *Faramea* (fig. 3).

These observations were partly checked upon long-styled material collected in Paraná by P. Dusén in 1908 (collection no. 7014) which also shows small, smooth grains.

The measurement, with a micrometer eyepiece, of 100 grains from each kind of flower gave the following results:

	Mean diameter	σ
Short styled (Weir)	23.4 units	±1.3
Long styled (Dusén)	16.4 units	±0.84

The ratio here is 1.43 : 1. This is in remarkably close agreement with the measurements by Fritz Müller who states in his paper that the ratio is as $\frac{1}{12}$th mm. to $\frac{1}{18}$th mm. (= 1.50 : 1).

An inspection of the preserved flowers shows that rotation of the anthers in the short-styled flowers takes place exactly as described by Müller and that the stigmata correspond with his descriptions. Consequently there can be no doubt at all that Müller's *"Faramea"* is *Rudgea jasminoides* Muell.-Arg. and that morphological pollen-dimorphism exists in the manner he described.

The possibility of dioecism was still not eliminated, however. It remained a possibility that the small, smooth grains from the long-styled flowers were non-functional. On the other hand, staining of the grains with hot aceto-carmine showed that both the spiniferous and the smooth grains were filled with cytoplasm containing a generative nucleus and a tube nucleus and seemed likely to have been functional. The ultimate test is the investigation of fruit-formation. Should fruits be formed by both long-styled and short styled trees then heterostyly must exist rather than dioecism. Unfortunately it was not possible to decide this question from the available herbarium material.

At the suggestion of Mr. N. Y. Sandwith, the author wrote to Dr. P. Raulino Reitz, Director of the Herbario "Barbosa Rodrigues" in Fritz Müller's home town of Itajahy (or Itajai). Dr. Reitz was very willing to collaborate and on Nov. 16, 1954 wrote saying, "In my last excursions I have observed 14 short-styled *Rudgea jasminoides* trees with mature fruits and many long-styled with fruits."

In January 1955 vouchers consisting of nine collections by Drs. Reitz and Klein from the neighbourhood of Itajahy were received. These consist of eight specimens of short-styled trees and one of long-styled. All were bearing fruit when collected and from the anthers of young flowers it was possible to make pollen preparations agreeing in every way with those described previously.

Thus the story is complete; pollen dimorphism is as striking in the heterostylous *Rudgea jasminoides* as it is in any member of the Plumbaginaceae and it is related to a similar incompatibility (outbreeding) system. Parallel evolution has occurred in the two families.

The Wider Implications

Darwin (1877, p. 135) quotes Fritz Müller as saying that *Rudgea eriantha* Benth. (now known as *R. coriacea* (Spreng.) K. Sch.) is heterostyled. Examination of herbarium material of this quite well-defined species has shown that it is indeed so and, although it was not noticed by Müller in this case, the pollen dimorphism is to be seen and it is likely that the phenomenon is widespread at least within the genus. Spiniferous pollen grains are, however, rare in the Rubiaceae; prior to this study of *Rudgea* they were known only from the tribe Spermacoceae (Erdtman, 1952) while the non-aperturate condition is also unusual. Although these features add to the certainty of the re-identification of Müller's material, they mean that pollen dimorphism, which must now be looked for in all heterostylous members of the family, is unlikely to involve the same patterns of exine-sculpture if it is found outside the genus *Rudgea*.

The presence or absence of spines on pollen grains is a major character in pollen morphology and the magnitude of the difference between the two pollen forms here is as great as that found between genera or higher taxa in many cases. It is interesting to the evolutionist, therefore, to reflect that if the genetics of heterostyly are the same here as prevail elsewhere, the difference is probably due to the alternative allelic states of a single gene.

The potential importance of the present demonstration for phytogeography is considerable. If the existence of such easily recognisable pollen-dimorphism can be established in a widespread tropical arboreal genus or group of genera the way is open for the investigation of just those phytogeographical and evolutionary problems which cannot be attacked with the

aid of the dimorphic Plumbaginaceae, for the latter are concentrated in maritime and arid regions, most often outside the tropics. Dimorphism and derived monomorphism in trees which are components of the forests of the tropics at the present day may provide information of the greatest value in the study of the history of these formations, where the fossil and quasi-fossil record is virtually non-existent.

Summary

The evolution of dimorphism in the pollen and stigmata of members of the Plumbaginaceae has been followed by a return, in some cases, to monomorphism. This has provided an extremely useful tool for the elucidation of genetical, genecological, phytogeographical and evolutionary problems. In the search for a comparable situation in other families, the neglected and ambiguous account of pollen dimorphism in *"Faramea"* described by Fritz Müller and accepted by Charles Darwin has been reinvestigated. Examination of the pollen of genuine *Faramea*, the tracing of Müller's herbarium material and the study of new collections has shown that *Rudgea jasminoides* (Cham.) Muell.-Arg. (Rubiaceae) is the species involved and that pollen dimorphism associated with heterostyly has evolved in the Rubiaceae in a manner quite comparable with that in the Plumbaginaceae.

The difference between the two pollen forms of a single species of *Rudgea* is as great as that which distinguishes some genera or higher taxa yet it may have a simple genetical basis. Occurring as it does in a genus of woody tropical plants (and possibly being present in related genera with a similar habit), this dimorphism may be an important aid to the study of the history and distribution of tropical forest floras, of which there is little fossil record.

Acknowledgments

It would not have been possible to investigate this problem without the generous help of the staff of the Herbarium of the Royal Botanic Gardens, Kew, England. In particular, I should like to thank Dr. W. B. Turrill, the Keeper, and Messrs. N. Y. Sandwith, A. A. Bullock and F. Ballard. International co-operation with Dr. P. Raulino Reitz set the seal upon the success of the study and I offer him my sincere appreciation.

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POPULATION VARIABILITY, HYBRIDIZATION, AND INTROGRESSION IN SOME SPECIES OF *OPHRYS*

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Introduction

One of the most remarkable genera of Orchidaceae in the temperate regions is *Ophrys*. This genus, which is distributed chiefly through the Mediterranean region of southern Europe, southwestern Asia, and North Africa, contains many species which resemble each other very closely in general habit and in vegetative characteristics, but which differ in respect to the shape of the flower, particularly the labellum, which usually possesses an intricate pattern of form and color. As was determined many years ago by Pouyanne (1917; Correvon et Pouyanne, 1916, 1923), the distinctive structure of the labellum of each species of *Ophrys* is associated with its peculiar method of cross pollination, which is accomplished by male bees through the process known as pseudocopulation. The bee is attracted to the flower because of a general resemblance in form and color to the female of its species, and particularly because of a distinctive odor. Upon reaching the labellum, he is aroused to copulatory action by the stimulus of the hairs on the labellar surface, and during his activity, pollinates the flower. The recent work of Godfery (1925, 1927b, 1930), Kullenberg (1948, 1950, 1952), and others has served to verify the earlier observations of the French workers in every important point, and has added many significant new facts about the process.

Among the Orchidaceae of the Mediterranean region, *Ophrys* occupies an unusual position. Most of the other genera (*Serapias, Anacamptis, Barlia, Orchis*) are closely related to each other, and intergeneric hybrids between them are known (Godfery, 1927; Keller and Soo, 1930–1938). *Ophrys*, on the other hand, is well separated from all of these genera, and taxonomic problems arise only within the genus itself. These problems are, however, rather numerous, since many of the species are highly polymorphic, and occasional hybrids between most of the species have been described (Keller and Soo, 1930, 1931). The possibility exists, therefore, that the polymorphism of *Ophrys* species could be due at least partly to the effects of interspecific hybridization, as has been postulated by Anderson (1953) for many species of higher plants. There are, however, no known examples of hybrid swarms, in spite of the fact that two, three, or even four species of *Ophrys* may be found growing together in the same habitat, and that they bloom at the same season. The F_1 hybrids usually occur as solitary or a a few individuals, surrounded by large numbers of plants of the parental species. The absence or rarity of hybrid swarms is particularly remarkable when one realizes that the F_1 hybrids produce an abundance of normal appearing pollen.

Unfortunately, the problem of the role of hybridization in the origin of intraspecific polymorphism in *Ophrys* is difficult to attack by direct genetic experiments, since the species of this genus are very difficult to raise in the greenhouse, and the time elapsed from seed to flowering is long. For this reason, indirect methods for estimating the effects of natural hybridization in populations, such as those developed by Anderson (1949, 1953) could help greatly in solving this problem.

With this possibility in mind, the present authors studied a population of *Ophrys lutea* Cav. and *O. fusca* Link, in the vicinity of Algiers. The exact locality was on the Route Nationale Alger-Blida, along the ravine of Oued El Kerma, at Baba Ali. At this locality, an open maquis of *Cistus* spp. and other shrubs, there were hundreds of plants of both *O. lutea* and *O. fusca,* while the junior author found by careful search nine plants intermediate between the two species. Two of these corresponded with x *O. Gauthieri* Lièvre (1922), which is generally recognized as the natural F_1 hybrid between *O. fusca* and *O. lutea,* and is known from several localities in southern Europe and North Africa. The other seven corresponded morphologically with *O. Murbeckii* (*O. lutea* subsp. *Murbeckii* (Fleischmann) Soo), a type which is widespread in North Africa (Keller and Soo, 1930).[1] From this population, 30 individuals each of *O. fusca* and *O. lutea,* plus the 9 intermediates, were collected and measured. Later, the senior author with M. Louis Faurel made collections of *O. Murbeckii* from three localities in the mountains of east central Algeria; the Col de Talmet, in the Belezma massif west of Batna, at an altitude of 1,700 m; the Djebel Mansourah, above Mansourah-les-Bibans, at 1,100–1,200 m altitude; and the Djebel Choukchot, northwest of M'sila, at 1,400–1,500 m altitude. In these localities, neither *O. fusca* nor *O. lutea* was seen, and they have not been recorded by other botanists from these mountains. On the other hand, *O. Murbeckii* was associated with *O. atlantica* Munby on both the Djebel Mansourah and the Djebel Choukchot. For these reasons, a comparison between the population found near Algiers and those from the mountains seemed desirable, although no natural hybrids were seen in the latter localities. We therefore studied 16 specimens of *O. Murbeckii* from the Col de Talmet, 10 specimens from the Djebel Mansourah, and 10 from the Djebel Choukchot in the same manner as the specimens from the vicinity of Algiers. The following measurements were made:

- a1, a2; length and width of uppermost outer tepal.
- b1, b2; length and width of a lateral outer tepal.
- c1, c2; length and width of a lateral inner tepal.
- e1, e2; total length and width of the labellum.
- f1; length of a lateral lobe of the labellum.
- f2; length of the middle lobe of the labellum.
- f3; width of the middle lobe of the labellum.
- f4, f5; maximum length and width of the papillate spot of the labellar epithelium.
- f6, f7; maximum length and width of the pilose dark area of the labellum.

In addition to those characters which could be measured, three characters were estimated visually; shape of the apex of the lateral tepals, curvature of the margin of the labellum, and angle of reflexion of the labellum at the epichiloid furrow.

After the measurements were obtained, the fact became evident that the difference between *O. lutea* and *O. fusca* in respect to all linear dimensions is not great and that much overlapping exists in most of them. For this reason, and because the differences between the species appeared to be more conspicuous in respect to the shape of the structures rather than their size, the following ratios were obtained:

- a 1:2; length: width of uppermost outer tepal.
- b 1:2; length: width of a lateral outer tepal.
- c 1:2; length: width of a lateral inner tepal.
- e 1:2; length: width of labellum.
- f 1:2; length of lateral lobe: length middle lobe of labellum.

[1] The use of the specific epithet *O. Murbeckii* is justified by the results of the present study, as is explained below.

f7 : e2; width pilose dark area : width labellum.

f5 : e2; width papillate spot : width labellum.

These measurements and estimates were combined according to the methods of Anderson (1949, 1953) to form a pictorialized scatter diagram of each population, and to give a hybrid index value for each specimen.

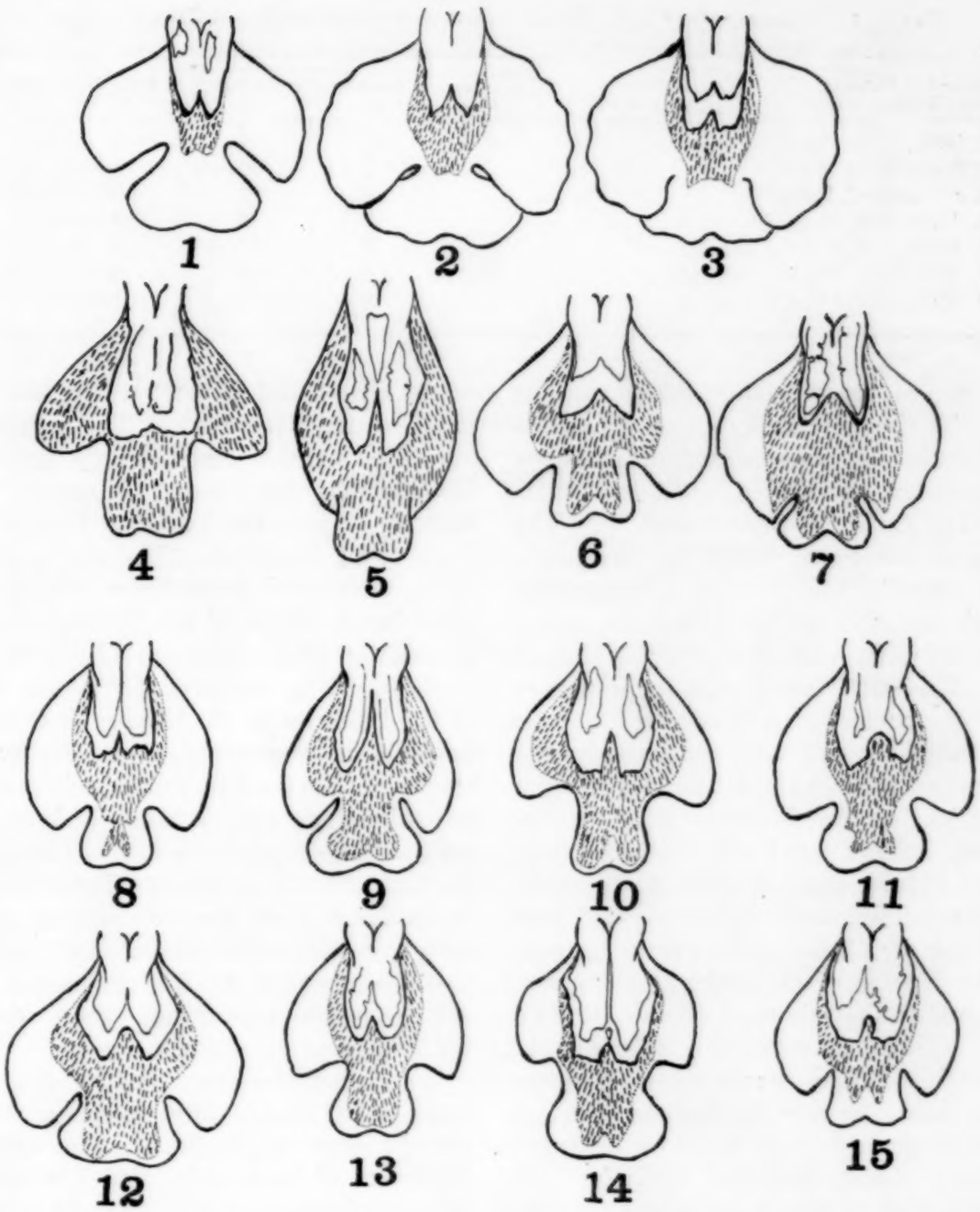
Differences in Individual Characters

The means and extreme values for eight representative linear dimensions are given in table 1. Due to the small number of individuals in some of the populations, and because the distribution of the variants did not usually follow the normal curve, the calculation of the standard deviation of the mean was not considered to be worthwhile. For the three populations of *O. Murbeckii* from the Hodna Mountain localities the width of the papillate spot of the labellar epithelium was not obtained.

When the values for *O. lutea* are compared with those for *O. fusca,* the first differences noted are in the breadth of tepals and labellum; all of these structures being narrower in *O. fusca* than in *O. lutea.* These differences are, however, not great, and there is much overlapping. Much more striking differences exist in the shape and markings of the labellum, as can be seen in figures 1 to 5. In *O. lutea,* the labellum has two broad, rounded lateral lobes, and a large middle lobe, which flares prominently from a deeply constricted base, between two deep sinuses. The surface of the lobes is glabrous and yellow in color. In the region between the lobes and toward the base of the labellum is a dark, shining, papillate spot or blotch, surrounded by an area which is dark and pilose. In *O. fusca,* the lateral lobes of the labellum are less rounded, but longer, measuring from the base of the labellum, while the middle lobe is smaller, and little or not at all constricted at the base, so that the sinuses

Table 1. *Mean and extreme values for eight representative dimensions of floral parts*

	Uppermost outer tepal		Lateral inner tepal		Labellum length (e 1)	Labellum width (e 2)	Length middle lobe (f 2)	Width papillate spot (f 5)
	Length	Width (a 2)	Length (c 1)	Width (c 2)				
O. lutea, Baba Ali	9.0–*10.9*–13.5	6.0–*7.4*–9.0	6.0–*7.6*–10.0	2.0–*2.9*–4.0	11.5–*13.3*–16.0	15.0–*16.6*–21.0	4.5–*6.3*–9.5	3.0–*4.3*–6.0
O. fusca, Baba Ali	8.5–*10.7*–12.0	5.5–*6.6*–8.0	5.5–*7.2*–9.5	1.5–*2.2*–3.0	11.5–*14.4*–21.0	11.0–*12.4*–14.5	4.0–*5.3*–9.5	5.0–*7.0*–8.5
O. x Gauthieri, Baba Ali	9.5, 9.5	6, 6.5	6.5, 8	2.5, 3.0	12.5, 14.0	12.0, 14.5	4.0, 5.5	3.5, 4.5
O. Murbeckii, Baba Ali	9.0–*9.8*–10.5	5.0–*6.0*–6.5	6.0–*7.0*–8.0	2.5–*2.7*–3.5	10.5–*11.6*–12.5	11.0–*11.9*–13.5	2.0–*3.1*–3.5	3.0–*4.1*–5.0
O. Murbeckii, Col du Talmet	7.0–*8.1*–9.5	5.0–*5.4*–6.5	4.5–*5.4*–6.5	1.0–*1.7*–2.0	9.5–*10.4*–12.0	8.5–*9.2*–10.5	3.0–*3.8*–4.5	—
O. Murbeckii, Dj. Mansourah	7.0–*8.4*–9.5	4.5–*5.6*–6.5	5.0–*5.3*–6.5	1.0–*1.7*–2.5	9.0–*9.8*–11.0	8.0–*9.4*–10.5	2.5–*3.1*–3.5	—
O. Murbeckii, Dj. Choukchot	6.5–*7.8*–9.0	4.5–*5.0*–5.5	4.5–*5.1*–6.5	1.0–*1.6*–2.0	9.0–*9.9*–12.0	8.5–*9.3*–10.0	2.5–*3.2*–4.0	—



FIGS. 1 to 15. Labellar shapes, × 2. Figures 1 to 3, *Ophrys lutea;* fig. 4, *O. fusca,* lip spread out; fig. 5, *O. fusca,* lip in natural position; fig. 6, *O. Murbeckii,* from Baba Ali; fig. 7, *O.* × *Gauthieri,* from Baba Ali; figs. 8–15, *O. Murbeckii,* various plants from the Hodna Mountain localities, showing the range of variation present.

between the middle and lateral lobes are much shallower and more open. The shining, papillate blotch at the base of the labellum is much larger than in *O. lutea,* and the pilose, dark area extends over the entire labellum.

The putative hybrid, x *O. Gauthieri* Lievre, has tepals and a labellum about the same length as in *O. lutea* and *O. fusca,* and intermediate between the two in width. The lateral lobes of its labellum are less rounded (fig. 7), while the middle lobe is relatively small, and intermediate in shape between *O. lutea* and *O. fusca.* The papillate blotch is also intermediate in size, while the pilose area extends to the apex, but not the lateral margins of the middle lobe, and covers about one half of the lateral lobes of the labellum. The labellum illustrated, drawn

TABLE 2. *Distribution of values for a1 : a2 (length/width of uppermost outer tepal)*

Numerical Value Index Value	1.0–1.2 1	1.3–1.4 1	1.5–1.6 2	1.7–1.8 3	1.9–2.1 3
O. lutea, Baba Ali	3	14	9	4	
O. fusca, Baba Ali		7	10	10	3
O. x Gauthieri, Baba Ali			2		
O. Murbeckii, Baba Ali			3	4	
O. Murbeckii, Col du Talmet	1	5	7	3	
O. Murbeckii, Dj. Mansourah	1	3	2	3	1
O. Murbeckii, Dj. Choukchot		5	2	1	2

from one of the two plants found wild at Baba Ali, is similar to that of the artificial hybrids of this combination made by F. Denis, and illustrated by Keller (1931, vol. III, Tafel 19, figs. 2 and 3). The principal difference is that in these artificial hybrids the sinuses between the lateral and the middle lobes are much more open than in the putative natural one. This difference is not surprising in view of the fact that forms of *O. lutea* exist with sinuses that are considerably more open than in the forms occurring at Baba Ali; such a flower is illustrated by Keller, Tafel 8, fig. 1. The exact parent of the hybrid made by Denis is, apparently, not illustrated, but it may well have been like the *O. lutea* of Keller's figure 1.

The flowers of *O. Murbeckii* are usually smaller than those of *O. lutea* and *O. fusca*. This is especially true of the mountain populations; the seven specimens from Baba Ali are intermediate in size between these and *O. lutea* or *O. fusca*. In tepal shape, they are variable, with the range of variation extending over the total range of both *O. lutea* and *O. fusca* (tables 2, 3). The shape of the labellum, on the other hand, is more nearly like that of *O. fusca* (table 4). The range of variation in the length-width ratio of labellum for the Hodna Mountain collections is just the same as that of *O. fusca* from Baba Ali. The seven plants of *O. Murbeckii* from Baba Ali, on the other hand, are more nearly intermediate in labellar shape between *O. fusca* and *O. lutea*. The shape of the lateral lobes of the labellum in *O. Murbeckii* is intermediate between those of the F_1 hybrid and of *O. fusca*. The middle lobe, on the other hand, is very variable in both size and shape (figs. 6, 8–15). The dark, papillate blotch is intermediate in size, as in the F_1, but the surrounding pilose area is considerably smaller, and variable in shape, so that the flowers have a distinctly yellow appearance when seen in the field.

Two other characteristics by which *O. lutea* and *O. fusca* differ from each other are the position of the labellum and the curvature of its margin. In *O. lutea*, the labellum is more or less strongly reflexed downward, while in *O. fusca* it is held in a horizontal position. The margin of the

TABLE 3. *Distribution of values for c1 : c2 (length/width of lateral inner tepal)*

Numerical Value Index Value	1.8–2.2 1	2.3–2.7 1	2.8–3.3 2	3.4–3.8 3	3.9–4.3 3	4.4–5.5 3
O. lutea, Baba Ali	5	14	16	5		
O. fusca, Baba Ali		6	12	7	2	3
O. x Gauthieri, Baba Ali		1	1			
O. Murbeckii, Baba Ali	1	4	1	1		
O. Murbeckii, Col du Talmet		3	7	2	3	1
O. Murbeckii, Dj. Mansourah	1	1	4	2	1	1
O. Murbeckii, Dj. Choukchot	1	1	5	1	1	1

TABLE 4. *Distribution of values for e1 : e2 (length/width of labellum)*

Numerical Value / Index Value	.70–.79 1	.80–.89 2	.90–1.04 3	1.05–1.19 4	1.20–1.50 5
O. lutea, Baba Ali	13	17			
O. fusca, Baba Ali			4	17	9
O. x Gauthieri, Baba Ali			2		
O. Murbeckii, Baba Ali		2	4	1	
O. Murbeckii, Col du Talmet				14	2
O. Murbeckii, Dj. Mansourah			2	8	
O. Murbeckii, Dj. Choukchot			2	7	1

TABLE 5. *Distribution of values for f1 : f2 (length lateral lobe/length middle lobe of labellum)*

Numerical Value / Index Value	1.0–1.7 1	1.8–2.3 2	2.4–2.8 3	2.9–5.5 3
O. lutea, Baba Ali	22	8		
O. fusca, Baba Ali	5	17	8	
O. x Gauthieri, Baba Ali		1	1	
O. Murbeckii, Baba Ali		2	1	4
O. Murbeckii, Col du Talmet	5	6	5	
O. Murbeckii, Dj. Mansourah	1	3	3	3
O. Murbeckii, Dj. Choukchot	1	4	4	1

labellum in *O. lutea* is concave, so that its edge forms a sort of gutter; while in *O. fusca* it is reflexed, so that the lateral lobes are bent under and are only partly visible in a surface view of the labellum (figs. 4, 5). In both of these characteristics the F_1 hybrids and *O. Murbeckii* show varying degrees of intermediacy.

Tables 2 to 8 show the distribution of values for certain critical ratios and the other characteristics mentioned in the description. For some of these characteristics, the range of variation in *O. lutea* overlaps that in *O. fusca,* but in respect to length/width of labellum (table 4), width pilose dark area/width labellum (table

TABLE 6. *Distribution of values for f7 : e2 (width of pilose dark area/width labellum)*

Numerical Value / Index Value	.20–.29 1	.30–.39 1	.40–.49 2	.50–.59 2	.60–.79 3	.80–.99 3	1.0 4
O. lutea, Baba Ali	4	22	4				
O. fusca, Baba Ali							30
O. Gauthieri, Baba Ali				2			
O. Murbeckii, Baba Ali			3	3	1		
O. Murbeckii, Col du Talmet			3	7	6		
O. Murbeckii, Dj. Mansourah			1	2	6	1	
O. Murbeckii, Dj. Choukchot			2	7	1		

TABLE 7. *Distribution of values for f5 : e2 (width papillate spot/width labellum)*

Numerical Value / Index Value	.17–.29 1	.30–.42 2	.43–.55 3	.56–.68 4
O. lutea, Baba Ali	26	4		
O. fusca, Baba Ali		1	11	18
O. x Gauthieri, Baba Ali	1	1		
O. Murbeckii, Baba Ali	2	5		

TABLE 8. *Distribution of values for g (curvature of margin of labellum)*

Type of curvature	Concave	Flat	Convex sloping downward	Convex reflexed
Index Value	1	2	3	4
O. lutea, Baba Ali	16	14		
O. fusca, Baba Ali			23	7
O. x Gauthieri, Baba Ali		1	1	
O. Murbeckii, Baba Ali		3	4	
O. Murbeckii, Col du Talmet	7	7	2	
O. Murbeckii, Dj. Mansourah		10		
O. Murbeckii, Dj. Choukchot	5	3	2	

6), width papillate spot/width labellum (table 7), and curvature of margin of labellum (table 9) the values for the two species are entirely distinct, and do not overlap.

In all of the characteristics studied, both the suspected F_1 hybrids and *O. Murbeckii* are intermediate between *O. lutea* and *O. fusca*. For every character recorded there are some specimens of *O. Murbeckii* which agree in their values with the putative F_1 hybrid, but for some characters, such as length/width of labellum and its degree of reflexion, the mean and modal values for *O. Murbeckii* differ recognizably from the values for the F_1. Because of the small number of individuals available, statistical tests could not be applied to these samples. The values for the three samples from the Hodna Mountains (Col de Talmet, Djebel Mansourah, Djebel Choukchot) are in general agreement with each other, and it seems likely that larger samples of these populations would show that they differ very slightly from each other if at all. The modal values for the seven specimens of *O. Murbeckii* from Baba Ali are mostly different from those of the Hodna Mountain samples, and tend to be more like those of the putative F_1 hybrid, *O.* x *Gauthieri*.

The opinion of Keller and Soo (1930), that *O. Murbeckii* is a subspecies of *O. lutea*, is not supported by the present study. As shown in tables 2 to 9, *O. Murbeckii* is more like *O. lutea* than *O. fusca* in three of the eight characters shown in these tables (shape of outer tepals, width of papillate spot, curvature of margin of labellum), but more like *O. fusca* in respect to the other five. Furthermore, in the regions of Algeria where *O. lutea* and *O. Murbeckii* are sympatric, there is no evidence of extensive intergradation between them. Since *O. Murbeckii* in the sum total of its characters is just as different and distinct from *O. lutea* as it is from *O. fusca*, it is best regarded as an independent species closely related to the other two.

Both the field observations and the distribution patterns for the individual characteristics given in the tables present the

TABLE 9. *Distribution of values for h (reflexion of labellum)*

Degree of reflexion	80–90°	ca. 45°	18–20°	Horizontal
Index Value	1	1	2	3
O. lutea, Baba Ali	8	13	9	
O. fusca, Baba Ali			15	15
O. x Gauthieri, Baba Ali		2		
O. Murbeckii, Baba Ali		4	3	
O. Murbeckii, Col du Talmet		8	3	5
O. Murbeckii, Dj. Mansourah		3	3	4
O. Murbeckii, Dj. Choukchot		5	4	1

following general picture. *Ophrys lutea* and *O. fusca,* as they occur in the vicinity of Algiers, are sharply distinct, easily recognizable species, and their populations are not particularly variable. They form occasional natural hybrids, but these plants are easily distinguished in the field from both parents, and hybrid swarms are unknown. Another type of plant intermediate between *O. lutea* and *O. fusca* is *O. Murbeckii.* This type may occur as scattered specimens in large colonies of *O. lutea* and *O. fusca,* as at Baba Ali, or as well developed populations apart from the two latter species. Most of these populations of *O. Murbeckii* are geographically and ecologically isolated from *O. lutea* and *O. fusca,* but according to M. Louis Faurel of the University of Algiers (oral comm.) populations of *O. Murbeckii* exist within the geographic ranges of the two other species. The geographic range of *O. lutea* and *O. fusca* covers the entire Mediterranean basin; that of *O. Murbeckii* is confined to Morocco, Algeria, and Tunisia.

Character Association, Hybridization, and Introgression

Anderson (1953) has recently stated the hypothesis that all hereditary variation in natural populations of higher plants may be the result of past hybridization and introgression, i.e., back crossing from the F_1 hybrids to the parental types accompanied by selection for certain back cross genotypes. He has distinguished two types of introgression, sympatric and allopatric, depending upon whether or not the introgressant genotypes occur in the same populations as the non-introgressed individuals of the parental species. In the genus *Ophrys,* one might expect to find both sympatric and allopatric introgression, for the following reasons. First, the genus contains many, closely related species which are sympatric to a greater extent than is usual for closely related species of the same genus. Second, many natural hybrids between species are known, and several of these have been duplicated by artificial hybridization (Keller and Soo, 1930–1931). Third, the chromosome numbers of all species which have been counted are the same, (Shimoya and Ferlan, 1952 and ined.), and pollen fertility of the hybrids is high, according to personal observations of the writers. Nevertheless, the superficial impression given by the populations which the writers have seen is not that of hybrid swarms or even of introgressed populations, but rather of distinct species. Each species appears to have its own pattern of variation, which bears little or no relationship to the variation patterns found in the species with which it is associated.

As Anderson has pointed out, however, the effects of introgression are often so subtle that they cannot be detected without the use of special techniques of recording observations. These techniques, designed to show simultaneously the variation in respect to several characters, are the hybrid index and the pictorialized scatter diagram. The results of their application to the populations concerned are as follows.

For computing the hybrid index, values were assigned to the eight characters shown in tables 2 to 9, as indicated on those tables. In respect to shape of outer and inner tepals, ratio of labellar lobe lengths, and reflexion of labellum (tables 2, 3, 5, 9) the overlapping between the species is so great that these characters have relatively little diagnostic value; hence only three index values (1, 2, 3) were assigned for each of these four characters. The length-width ratio of the labellum (table 4) shows a relatively large amount of variation for the group as a whole, and the two parental species are well separated from each other in respect to this character. For this reason, the numerical values 1 through 5 were assigned to this character, while the values 1 through 4 were assigned to the remaining three characters. Whenever possible, the values were assigned in such a way

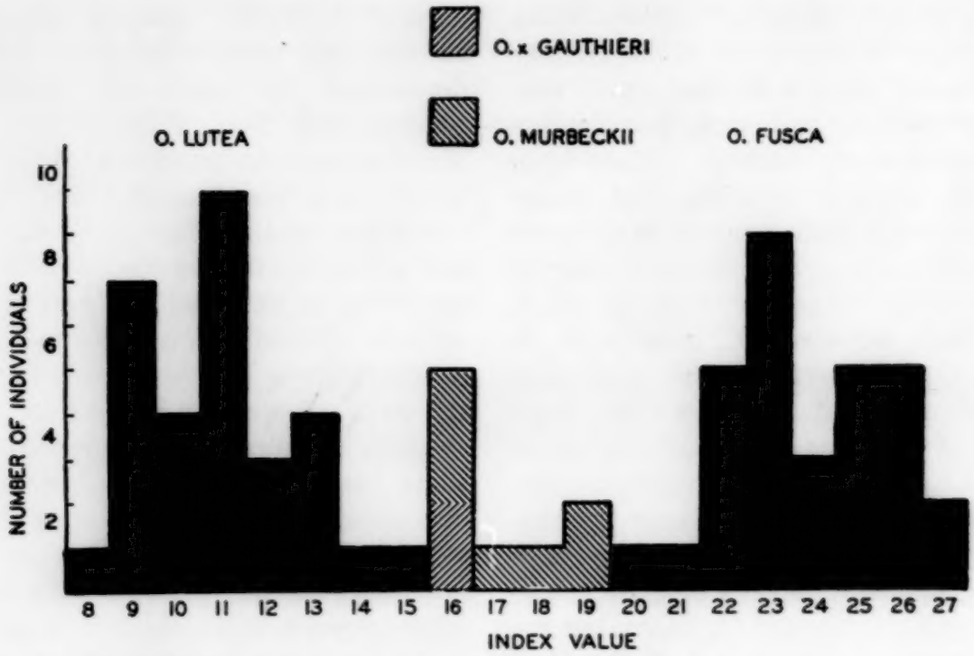


FIG. 16. Chart showing the distribution of hybrid index values in the population of *Ophrys lutea, O. fusca* and the intermediate forms collected at Baba Ali. Explanation in the text.

as to make the classes about equal in size for each value. For each character, low values were assigned to *O. lutea,* and high ones to *O. fusca.*

With this system of scoring, the lowest possible index value for the eight characters is 8, and the highest possible value is 29. The actual distribution of hybrid index values in the populations from Baba Ali is shown in figure 16. The great majority of the plants of *O. lutea* had values ranging from 9 to 13, while the values for *O. fusca* ranged mostly from 22 to 27. The two individuals of *O. lutea* with values of 14 and 15, as well as those of *O. fusca* with values of 20 and 21 might possibly be interpreted as introgressants, judging from their position on figure 18. On the other hand, this impression would disappear completely if the value for the individual having 15 were lowered by one point, and if that of the individual having a score of 20 were raised by one point. Slight changes such as this can easily be brought about by altering the basis on which index values are assigned. The general picture presented by figure 18, therefore, supports the first impression obtained from field study of the populations, and indicates that sympatric introgression is lacking.

In addition, figure 16 shows the intermediate position of both the putative hybrid, *O.* x *Gauthieri* and the seven individuals of *O. Murbeckii.* Comparable index values for the Hodna Mountain populations could not be obtained, since one of the measurements was not made on the fresh specimens, but index values computed on the basis of seven characters are similar to those computed for the same seven characters in the specimens of *O. Murbeckii* from Baba Ali. Some of the Hodna Mountain specimens had values high enough to overlap with the ranges of values for *O. fusca.* This does not mean, however, that these individuals were indistinguishable from *O. fusca,* since the combination of characters was in every case different. In particular, the dark spot and pilose area on the labellum in all plants of *O. Murbeckii* are equally or more like these characters in *O. lutea* than in *O. fusca.*

As a second test of introgression, a pictorialized scatter diagram was made to

represent the samples from Baba Ali (fig. 17). For the coordinates of this diagram, the characters selected are length/width ratio of the labellum and width papillose spot/width labellum. Five characters are represented by rays on the symbols, as indicated in the legend.

In the opinion of the writers, this diagram fulfills the prediction of Anderson (1953, p. 282), and shows "the comparative unimportance of hybridization, given natural populations in which hybridization is not an important factor." The symbols representing *O. lutea* are all in the lower right hand corner of the diagram, and are clearly not grouped along a regression line. Furthermore, the rays are distributed more or less at

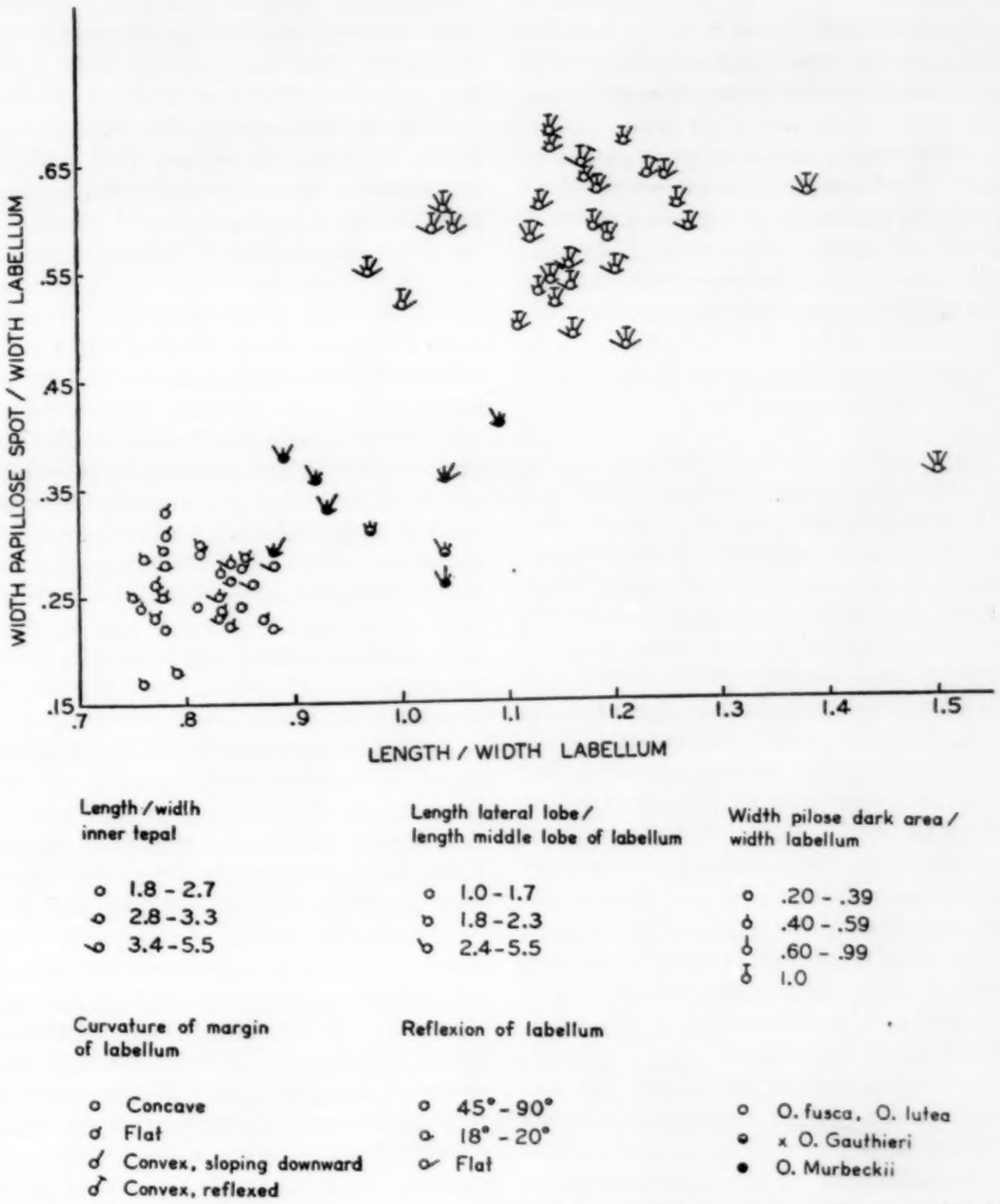


FIG. 17. Pictorialized scatter diagram representing the variation in respect to seven floral characteristics in the population at Baba Ali.

random with respect to the position of the symbols. The symbols representing *O. fusca* are all some distance away from those representing *O. lutea,* and likewise are not grouped along a regression line. The distribution of the rays, as in *O. lutea,* is at random with respect to the position of the symbols. The loose character association which Anderson considers to be diagnostic of introgression is not evident.

The intermediacy of both *O.* x *Gauthieri* and *O. Murbeckii* is evident from the position of the symbols representing these types, and from their possession of an intermediate number and length of rays. Furthermore, the specimens of *O. Murbeckii* do not show a graded series of transitions from *O. lutea* to *O. fusca,* but appear to form an independently varying group intermediate between the other two.

Effect of Floral Biology on Hybridization

The relation of floral biology to hybridization in *Ophrys* has been recognized by Kullenberg, who stated (1950, p. 16): "All the species within the genus are considered to be crossable with each other, but the specialized floral biology prevents this from happening to a large extent." Shimoya and Ferlan (1952) found that several species, including *O. lutea* and *O. fusca,* have the same chromosome number, $2n = 36$. The writers observed that the hybrids have normal appearing pollen, indicating that they should be capable of producing offspring, either through cross pollination with each other, or through back crossing to the parental species.

The results of the present study are paradoxical in relation to these theoretical expectations. In the region of Baba Ali, hybridization between *O. fusca* and *O. lutea* is rare, and in the population analyzed by the present authors the hybrids have not served as a means of altering the variation patterns of the parental species by means of introgression. On the other hand, a widespread, more or less well stabilized genetic entity, *O. Murbeckii,* which often occurs in regions away from the habitats of either *O. fusca* or *O. lutea,* nevertheless possesses a complex of morphological characters which are difficult to explain except as the result of hybridization between these two species.

In the opinion of the present writers, this situation can be explained by the known facts about the pollination of *O. fusca* and *O. lutea.* These species have been carefully studied by Pouyanne and Godfery in Algeria (Godfery, 1925, 1930) by Godfery (1927b) at Hyères, France, and by Kullenberg (1950) in Lebanon. These authors are agreed that both *O. fusca* and *O. lutea* are pollinated by male bees belonging to the genus *Andrena.* A bee is attracted to a flower by its odor, and alights on the flower in a position facing the base of the labellum. Contact with the hairs of the labellar surface immediately stimulates him to violent activity. He turns around, places the tip of his abdomen in the furrow at the base of the labellum, and moves it rapidly back and forth. During this activity the upper surface of the abdomen strikes the pollinia and removes them, stuck fast to its surface. The bee leaves the flower, still in a state of nervous excitation, and becomes attracted to another, which it visits in the same manner, thereby effecting cross pollination.

Published reports indicate that both the specificity and the effectiveness of this process are very variable. The odor of the flower differs in the two species. Kullenberg (1952) describes that of *O. lutea* as similar to farnesol, geraniol, or a mixture of these two compounds, while that of *O. fusca* is a mixture of farnesolic, citronellalic, and octyl alcohol components. The odor of *O. Murbeckii* (*"O. subfusca"*) is like that of *O. fusca.* At least some of the pollinators are sensitive to this difference. Godfery (1930) states that *Andrena fulvicrus* Kirby ♂, although strongly attracted to *O. fusca,* paid no attention to flowers of *O. lutea.* On the other hand, *A. nigro-olivacea* Dours. was

seen to visit both species. *Andrena senecionis* has been seen only on *O. lutea,* while *A. trimmerana* and *A. nigroaenea,* like *A. fulvicrus,* are, so far as is known, confined to *O. fusca.* The data available, therefore, suggest that the chances for cross pollination between *O. fusca* and *O. lutea* depend on the species of *Andrena* which are present.

To a human observer, Kullenberg, *O. Murbeckii* has the same odor as *O. fusca,* and therefore might attract the same bees. Since, however, no actual observations are recorded for this species, such an assumption is not justified. It is entirely possible that the sense organs of bees would discriminate between odors which to humans seem just alike. In addition, cross pollination depends upon stimulation of the bee after he reaches the flower, which is brought about by the hairs on the labellar surface. The shape and texture of these hairs appears to be very similar in *O. lutea, O. fusca,* and *O. Murbeckii,* but according to Kullenberg (1950) the type of movements, if any, which the bee makes depends upon the nature of the entire labellar surface, including general shape as well as nature and distribution of hairs and papillae. He records several instances of male hymenoptera which were attracted to various species of *Ophrys* by the scent of the flowers, but were stimulated to movements which did not resemble the prelude to copulation, and failed to dislodge the pollinia. The effectiveness of the specificity of a particular labellar structure cannot be determined except by careful and complete observations, which up to the present are lacking for *O. Murbeckii.* In fact, the ability of *Andrena nigro-olivacea* to pollinate both *O. lutea* and *O. fusca* is not definitely established.

Another fact, emphasized by both Pouyanne and Godfery and verified by the junior author, is that the number of flowers which become fertilized and form capsules full of seeds varies greatly from one population to another of *O. lutea* and *O. fusca* in Algeria. In some populations as little as 5 per cent of the flowers form capsules; while in others capsules have been seen on 70 to 80 per cent of the flowers. If the flowers are very numerous and the insects few, the chances for cross pollination are much less than in small populations of *Ophrys* which are situated near large colonies of *Andrena.* At Baba Ali the former situation was noted, and the expectation of fertilization would therefore be low.

The best explanation for the situation at Baba Ali is as follows. Cross pollination, even within the species *O. lutea* and *O. fusca,* is uncommon, and is carried out by those species of *Andrena* which confine their visits to one species of *Ophrys.* The two probable F_1 hybrids came about through a chance mistake by one of these pollinators, or through accidental cross pollination by another type of insect, such as the species of Coleoptera which occasionally visit these flowers (Kullenberg, 1950). The seven individuals of *O. Murbeckii,* on the other hand, may have entered the area from some larger population of this species situated in the vicinity. These intermediate individuals may be too different from both *O. lutea* and *O. fusca* to be visited by the normal pollinators of these parental species, and they are too few in number ever to be involved in the accidental visits which cause the initial hybridization. They are therefore considered to be sterile because of their floral structure, even though they would be fertile if pollinated artificially.

In Algeria, therefore, a very effective barrier of reproductive isolation exists between the completely sympatric species *Ophrys fusca* and *O. lutea* which is apparently due entirely to differences between them in floral structure and the selective behavior of the male bees which pollinate them.

The Origin of Species in *Ophrys*

In considering the second major problem raised by this study, namely the origin of *O. Murbeckii,* the following facts must

be noted. First, the form of its flowers is intermediate between *O. lutea* and *O. fusca* in many of their morphological characteristics, but is not the same as the supposed F_1 hybrid. Second, the species occurs not only as occasional individuals mixed with populations of *O. fusca* and *O. lutea,* but also abundantly in regions where both of these species are absent. In these regions, *O. Murbeckii* differs significantly from Algerian populations of *O. lutea* and *O. fusca* in the smaller size of its flowers. Since these areas are montane and relatively moist, the ecological preference of *O. Murbeckii* is apparently more mesophytic in character than that of either *O. fusca* or *O. lutea.* Third, *O. Murbeckii* is one of a series of variants related to *O. lutea* which possess a darker, more hairy labellum and other characteristics resembling *O. fusca.* Another of these forms is *O. lutea* subsp. *Melena* Renz (1928) of Greece. Fourth, although the chromosome number of *O. Murbeckii* is not known, its pollen grains are regular and of the same size as those of *O. fusca* and *O. lutea,* suggesting that it is not a polyploid.

It is hard to explain the intermediacy of *O. Murbeckii* on any other basis except the assumption that it is some sort of hybrid derivative. Its distribution, however, suggests that it is of ancient origin. The fact that the individuals collected at Baba Ali were well set off from those of the probable F_1 suggests that they did not originate through segregation of hybrids which occurred in this population, but that they entered the population from some larger colony of *O. Murbeckii* occurring elsewhere in the region of Algiers.

The following hypothesis would explain the situation. We must assume that the evolution of the floral types in *Ophrys* is closely associated with the origin and distribution of the species of Hymenoptera whose males pollinate their flowers by pseudocopulation. *Ophrys fusca* and *O. lutea* have doubtless existed side by side for thousands of years, and have during all of this time formed occasional interspecific hybrids. The vast majority of these hybrids, like the ones collected near Algiers, have been entirely sterile because they could not be pollinated. But from time to time there has arrived, either through migration or evolution, a type of hymenopteran with instincts adapted to pollinating these hybrids by pseudocopulation. When such rare events have occurred, the hybrids have produced segregating progenies. But among such progenies only those flowers were pollinated which attracted the species of male bee concerned. There was thus exercised a very strong selective pressure, stabilizing the progeny; both rendering it constant and causing it to deviate from the parental species as well as from the F_1 hybrid. Selection for different ecological preferences, such as greater mesophily, would also be effected by the ecological preferences of the species of hymenopteran concerned.

An alternative hypothesis would be that *O. Murbeckii* is the descendant of an ancient common ancestor from which *O. lutea* and *O. fusca* have diverged in different directions. There are several reasons for considering this hypothesis to be improbable. In the first place, *O. Murbeckii* has a more restricted distribution than either *O. lutea* or *O. fusca.* It occurs in a region with a relatively impoverished flora, which is generally considered to have been derived from more ancient centers farther to the east (Hagen, 1914). Secondly, both *O. lutea* and *O. fusca* are divided into a number of varieties or subspecies, some of which, like *O. Murbeckii,* are intermediate between the two species, while others vary in the direction of still different species. This suggests both that *O. lutea* and *O. fusca* are relatively old compared to *O. Murbeckii,* and that other hybrid derivatives have become stabilized during the evolution of the genus. Finally, the populations of *O. Murbeckii* in the Hodna Mountains, and probably elsewhere in Algeria, show a good deal of intra-popu-

lation variability as compared to *O. lutea* and *O. fusca*. This would not be expected of an ancient, relic type, but would occur in a relatively recent hybrid derivative which had not yet become completely stabilized.

In conclusion, we must mention the fact that similar situations probably exist in other sections of the genus *Ophrys*, such as in *O. arachnitiformis* and *O. obscura* of the Section Araniferae. In all probability, therefore, the evolutionary processes which have been responsible for the origin of *O. Murbeckii* have played important roles throughout the evolution of the genus. As in the present example, use of the diagrammatic methods of Anderson in other instances will aid in understanding the role of hybridization, selection, and other evolutionary processes throughout this remarkable genus.

Summary

Measurements were made of certain floral characteristics in thirty plants each of *Ophrys fusca* and *O. lutea*, of two probable interspecific hybrids, and of seven plants of *O. Murbeckii*, all collected in the vicinity of Algiers. Similar measurements were made of several plants of *O. Murbeckii* from other parts of Algeria. From these measurements, certain ratios were computed, and various ones of these characters were combined according to the methods of the pictorialized scatter diagram and the hybrid index, as devised by Anderson. The two parental species are most easily separated on the basis of certain ratios of measurements of the labellum, and are clearly distinguished by the combination of characters used on the pictorialized scatter diagram. In respect to all of these characters, the supposed F_1 hybrids are intermediate between their putative parents, but the plants of *O. Murbeckii* deviate from both parents in respect to certain characters, particularly the small size of the labellum. Neither the pictorialized scatter diagram nor the distribution of hybrid index values give any indication of introgression of genes from *O. fusca* into *O. lutea* or vice versa at the locality studied. Both the lack of sympatric introgression and the possible origin of *O. Murbeckii* as a hybrid derivative are explained on the basis of the peculiar and strong selective pressure exerted by the unusual mode of cross pollination effected by males of various species of Hymenoptera. There is good indication that interspecific hybridization has played a role in the evolution of forms of *Ophrys*, but that this role has been greatly modified by the selective action of the insect vectors.

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SEROLOGICAL LIGHT ON PORCUPINE RELATIONSHIPS

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The intriguing zoogeographic puzzle presented by the Old World and New World porcupines has recently aroused renewed interest (Wood, 1950; Lavocat, 1951a, b; Simpson, 1945, 1953). Do the porcupines all belong to one group which, in some mysterious way, managed to reach abodes on the two sides of the South Atlantic? Or are the American and African porcupines rather distantly related rodents which have come to resemble one another in many ways through parallel evolution on their respective continents (the view of the authors cited)? And what are the relationships of these porcupines to South American hystricomorph rodents that are not porcupines? We are indebted to Dr. Albert E. Wood for the suggestion that serological evidence might shed valuable light on these questions, the answers to which form the objectives of our investigation.

Previous serological studies of animals of known genetic relationship or of relationship clearly evident on morphological grounds have regularly resulted in confirmation of these relationships (Nuttall, 1904; Boyden, 1942; Moody, Cochran and Drugg, 1949, and references cited by these authors). This fact tends to instill confidence in the validity of relationship conclusions based on serological findings when the morphological data are inconclusive or subject to varied interpretation.

PORCUPINE RELATIVES AND RELATIONSHIPS

The order Rodentia is commonly divided into three suborders: Sciuromorpha, Myomorpha and Hystricomorpha (Simpson, 1945). Differing modes of attachment of the masseter muscle and modifications of the zygomatic arch induced by that muscle form a primary basis for this classification (Wood, 1947, 1950). Despite the general assumption that similar modifications of masseter and zygoma are indicative of genetic relationship, recent investigators have stressed the frequency of parallel evolution among rodents and have questioned this assumption (Wilson, 1949; Wood, 1947, 1950; Lavocat, 1951b).

Rodents placed in suborder Hystricomorpha have a portion of the masseter muscle arising on the side of the snout and passing through a greatly enlarged infraorbital foramen on the way to insertion on the mandible (Romer, 1945, fig. 371). Most of the modern rodents so characterized belong to the group that evolved in South America during the Tertiary: cavies, porcupines, agoutis, pacas, capybaras, chinchillas, and their relatives, including the migrant to North America, *Erethizon*, the North American porcupine. To these are added a few Old World forms, notably the porcupines of the family Hystricidae but also including the two African families, Thryonomyidae (cane "rats") and Petromyidae (rock "rats").

In the light of extensive parallel evolution among rodents are we warranted in concluding that all rodents having the hystricomorph type of zygomasseteric structure are closely enough related to justify inclusion in one suborder? The question is particularly cogent in view of the distributional problem presented by the hystricomorphs. They appeared in the Old World and in South America in Oligocene times but none occurred in North America until the Pliocene. Land bridges, rafting across the Atlantic ocean

and island-hopping via Antarctica have been evoked to explain how an ancestral hystricomorph could have passed from the Old World to South America early in the Tertiary. Wood (1950) discussed the whole question in detail and suggested that the Old World and New World hystricomorphs arose independently from Eocene rodents, probably members of the widespread family Paramyidae. Lower Eocene members of this family "include some forms which seem to be very close to being the common ancestors of all later rodents" (Wood, 1950). Although the paramyids are not hystricomorphs, Wood recorded that one Upper Eocene genus, *Rapamys,* had an enlarged infraorbital foramen "and had begun the 'hystricomorph' type of masseteric specialization." Lavocat (1951b) supported the view that the two groups of porcupines evolved independently, bringing to attention other striking instances of parallel evolution among rodents, some involving independent enlargement of the infraorbital foramen. The possession of quills by the two families appears to present no great obstacle to independent origin since the hair of other rodents, and of mammals that are not rodents, has developed into quills and spines of varying types.

In line with the idea that Old World and New World hystricomorphs arose independently from non-hystricomorph ancestors, Lavocat (1951a) divided the suborder into an Old World section, "Orthohystricognathes," and a New World section, "Parahystricognathes." Schaub (1953; according to Wood, 1955) placed the New World forms in an infraorder, "Nototrogomorpha," separate from the Old World forms. Finally Wood and Patterson went the whole way, creating "Suborder Caviomorpha" for the New World forms and reserving Suborder Hystricomorpha for the Old World ones (Wood, 1955).

In this connection the fact is of interest that the lice (Mallophaga) parasitizing Old World hystricomorphs are not directly related to those of South American forms (Vanzolini and Guimarães, 1955).

Antigens

Normal blood sera from the following rodents were used as antigens in the tests (classification from Simpson, 1945).

Suborder SCIUROMORPHA
- *Marmota monax* L., woodchuck

Suborder MYOMORPHA
- *Rattus norvegicus* Erxleben, albino rat

Suborder HYSTRICOMORPHA

Superfamily Hystricoidea
- *Hystrix* sp. L., African porcupine

Superfamily Erethizontoidea
- *Erethizon dorsatum* L., North American porcupine

Superfamily Cavioidea

Family Caviidae
- *Cavia porcellus* L., domestic guinea pig

Family Dasyproctidae
- *Dasyprocta* sp. Illiger, agouti

In addition, sera of man (Order Primates) and of the domestic rabbit (Order Lagomorpha) were tested with one antiserum.

We are indebted to the Serological Museum of Rutgers University and to Dr. I. H. Vincke, Le Médecin Directeur de la SERAM, Services Médicaux de la Province du Katanga, Belgian Congo, for African porcupine serum, to Clark Hinsdale, Richard Breen, Richard Burns and members of the University of Vermont Outing Club for American porcupine specimens, and to Peter Finlay for woodchuck specimens. The agouti serum was obtained from a male specimen imported from Colombia, South America.

Antisera

Four antisera were produced in male domestic fowl, two against American porcupine serum (*Erethizon*) and two against African porcupine serum (*Hystrix*). Each antiserum consisted of

pooled serum from two cocks each of which had received a total of 18 ml of porcupine serum inoculated in eight small subcutaneous injections over a period of a month or more.

Technique

In the form of precipitin test employed, dilutions of antigen were mixed with constant quantities of antiserum, the magnitudes of the resulting reactions being indicated by the degrees of turbidity developed. This turbidity was measured with a Libby Photronreflectometer (Libby, 1938). The technique employed in operation of the instrument was essentially similar to that of Baier (1947, 183–184).

For each test a series of doubling dilutions of antigen was prepared, using a 12 per cent solution (cf. Goodman, Wolfe and Norton, 1951; Watson, 1950) of sodium chloride buffered to neutrality (Evans, 1922). The first tube in the series contained one part of whole serum to four parts of diluent. 1.7 ml of each dilution was transferred to a reaction cell of the photronreflectometer. 0.3 ml of antiserum, diluted as experience with that particular antiserum dictated (usually 1:1 or 1:1.5), was added to each cell. This antigen-antibody mixture was incubated for 40 minutes at 37° C.

Each test yielded a series of turbidity determinations representing the magni-

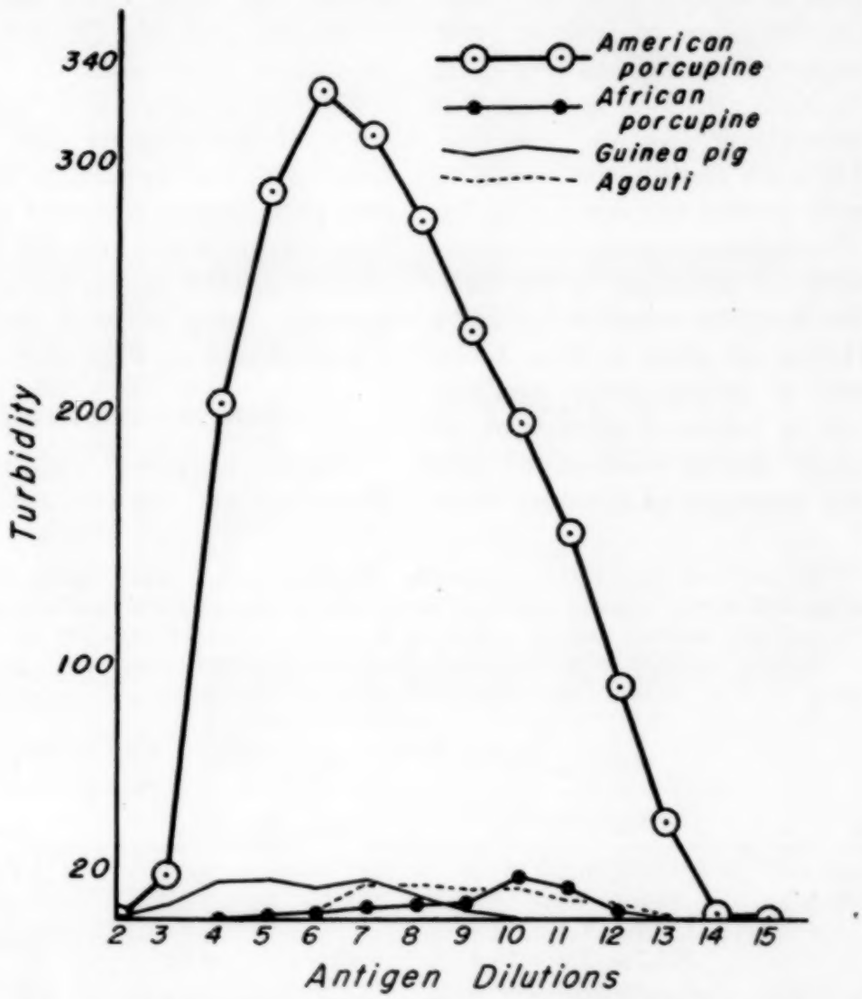

Fig. 1. Photronreflectometer reaction curves obtained with anti-American-porcupine serum III. Abscissa: reaction cell numbers, representing successive doubling dilutions of antigen. Ordinate: turbidity expressed in galvanometer units; readings converted to a reference-setting of 50.

TABLE 1. *Tests with two anti-American-porcupine* (Erethizon) *sera. Mean values are based on the summed ordinates of three or more reaction curves (except as noted) and are in terms of galvanometer units at a photronreflectometer reference-setting of 50. Each mean is accompanied by its standard error.*

Antigen	Antiserum II		Antiserum III	
	Mean	Percentage	Mean	Percentage
American porcupine	916.8 ± 5.4	100.0	2143.2 ± 11.8*	100.0*
African porcupine	124.0 ± 3.0	13.5	47.7 ± 7.7	2.2
Guinea pig	102.0 ± 0.6	11.2	76.5†	3.6†
Agouti	96.3 ± 0.9	10.5	64.5 ± 13.5*	3.0*
Woodchuck	—	—	0†	0†
Albino rat	87.3 ± 2.3	9.7	0†	0†
Domestic rabbit	43.7 ± 1.2	4.8	—	—
Man	24.0 ± 2.0	2.6	—	—

* Average of two tests.
† Reading from a single test.

tude of antigen-antibody reactions in a doubling series of antigen dilutions ranging from concentrations of antigen great enough to suppress precipitate formation (prozone) to concentrations so small that detectable precipitate was not produced. As is usual in such studies, the results of each test can be plotted as a curve (fig. 1). Summing the ordinates of such a curve gives a numerical statement of the magnitude of the complete reaction (tables 1 and 2). The means given in these tables are in terms of galvanometer readings converted to a reference-setting of 50 (Bolton, Leone and Boyden, 1948), and represent the averages of three or more complete repetitions of a test, except as noted. In some cases the quantity of antiserum available did not permit three repetitions of all tests.

Ease of comparison is facilitated if results of homologous and heterologous tests with an antiserum are converted into percentages, the total reading of the homologous test (i.e., the test with the antigen against which the antiserum was formed) being equated to 100 per cent (tables 1 and 2; Fig. 2).

RESULTS AND DISCUSSION

Results obtained with the two anti-*Erethizon* and the two anti-*Hystrix* sera

TABLE 2. *Tests with two anti-African-porcupine* (Hystrix) *sera. Mean values are based on the summed ordinates of three or more reaction curves (except as noted) and are in terms of galvanometer units at a photronreflectometer reference-setting of 50. Each mean is accompanied by its standard error.*

Antigen	Antiserum I		Antiserum II			
			Diluted 1:1.5		Diluted 1:1	
	Mean	Percentage	Mean	Percentage	Mean	Percentage
African porcupine	1031.5±10.7	100.0	1617.9±35.6	100.0	2121.5±127.1	100.0
American porcupine	85.0± 3.8	7.3	105.7± 3.6	6.5	(166.5)*	(7.8)*
Guinea pig	130.7± 1.2	12.7	—	—	51.2± 4.0	2.4
Agouti	106.7± 2.9	10.3	0‡	0‡	0†	0†
Woodchuck	—	—	—	—	0‡	0‡
Albino rat	44.7± 5.2	4.3	—	—	0‡	0‡

* Based on a single test and a partial repetition of it.
† Average of two tests.
‡ Reading from a single test.

agree in indicating but slight serological similarity between American and African porcupines (tables 1 and 2; fig. 2). As is always the case in such investigations, the various antisera manifested individuality of reaction with heterologous antigens, but the general trends were consistent. The maximum reaction of African porcupine serum with anti-*Erethizon* serum was of the order of 14 per cent of the homologous reaction, while the maximum reaction of American porcupine serum with anti-*Hystrix* serum was about 7 per cent. Because of variables in biologic tests of this sort, including, frequently, individuality of response to injected antigens on the part of antibody producers, small differences in percentage of weak heterologous reactions should probably be given little emphasis. Thus, the four heterologous reactions involving porcupine antigens (13.5, 2.2, 7.3, 6.5 per cent) may all be considered of the same "order of magnitude" relative to the 100 per cent symbolizing the homologous reactions of the four antisera.

The slightness of serological similarity between American and African porcupines is further emphasized by the reactions of these four antisera with sera of other hystricomorphs: guinea pig and agouti. The two anti-*Erethizon* sera reacted about as strongly with guinea pig and agouti sera as they did with African porcupine serum (antiserum II slightly less so, antiserum III slightly more so, but the differences are probably not significant; fig. 2). Anti-*Hystrix* serum I reacted more strongly with guinea pig and agouti sera than it did with American porcupine serum. Anti-*Hystrix* serum II was a narrowly specific reagent; it reacted somewhat more strongly with American porcupine serum than it did with guinea pig serum and it gave no reaction with agouti serum (fig. 2) or with

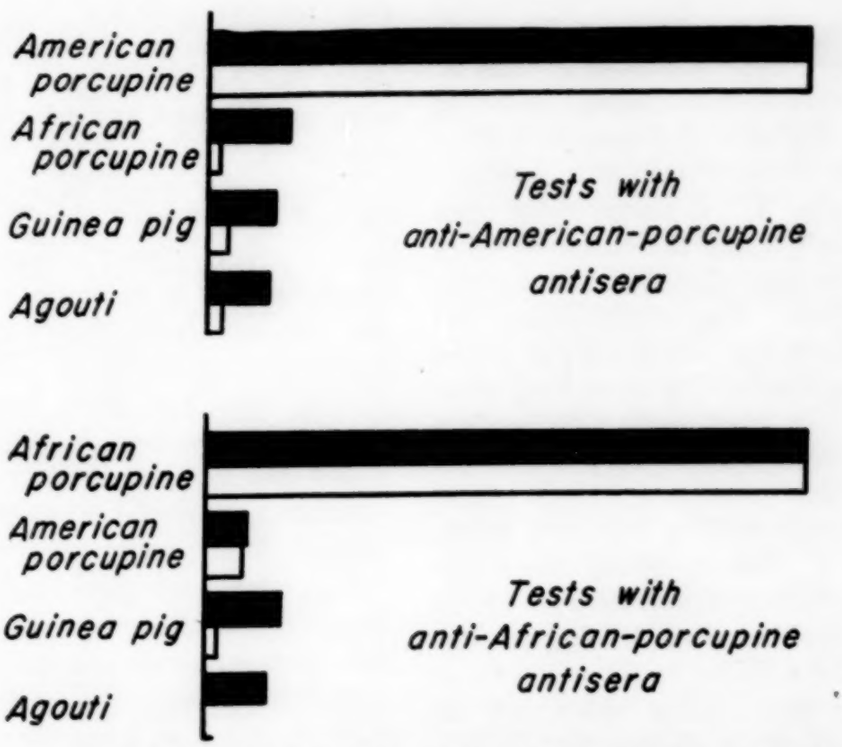

FIG. 2. Serological relationship values obtained with four antisera reacting with the sera of four hystricomorph rodents. Based on percentages given in tables 1 and 2. Upper half: tests with anti-*Erethizon* (American porcupine) sera II (black bars) and III (white bars). Lower half: tests with anti-*Hystrix* (African porcupine) sera I (black bars) and II (white bars).

the non-hystricomorph rodent sera tested (table 2). ("No reaction" should be interpreted quantitatively; there was no reaction under the conditions of the experment. If tests with undiluted antiserum had been practicable a reaction might well have been obtained. The small quantity of antiserum available precluded such tests.)

Taken as a whole the data give evidence that the American and African porcupines are as closely related to each other as either is to such other hystricomorph rodents as guinea pig and agouti, but no more so. Accordingly we note that the hystricomorphs investigated fall naturally into three groups, each one having but slight serological similarity to the other two: (1) non-porcupine hystricomorphs of South America, exemplified by guinea pig and agouti; (2) North American porcupine, *Erethizon;* (3) African porcupine, *Hystrix*. These three may be represented as the points of an approximately equilateral triangle (CHE, fig. 3). Figure 3 represents a few twigs of a branch of the "tree" of rodent evolution; it relates our serological findings on recent forms to the probable phylogenetic history that led to the characteristics now observed. The guinea pig, "C", is placed at the apex of a twig about which are clustered other twigs representing other South American hystricomorphs. The evolutionary line leading to the North American porcupine, "E", is shown as having been long separated from the lines leading to modern South American hystricomorphs that are not porcupines. Simpson (1945) states that the Erethizontoidea has been recognizable as a distinct group since the Oligocene (cf. also Wood, 1955). Hence our finding of marked serological dissimilarity of *Erethizon* to the other forms agrees with expectation based on the known history of the group.

The evolutionary line leading to point "H" in figure 3, the African porcupine, is shown as separating early from the lines leading to all the New World forms. The diagram thus accords with the view

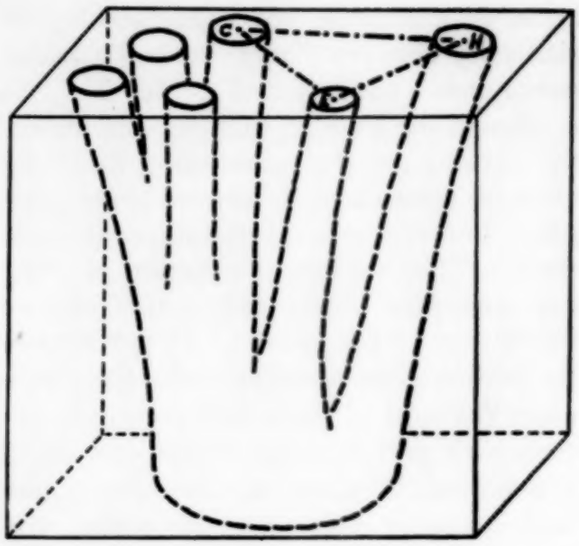

FIG. 3. Diagram showing relationships to each other or three recent hystricomorph rodents, together with representation of the probable phylogenetic history that led to the present diversity. "C", *Cavia* (guinea pig). "E", *Erethizon* (North American porcupine). "H", *Hystrix* (African porcupine). The vertical time axis extends from the Eocene to the present.

that the New World and Old World hystricomorphs are distantly related rodents that have undergone parallel evolution, as discussed in a previous section of this paper.

Despite the length of separation, lines leading to "C", "H" and "E" (fig. 3) are shown arising from a common branch, representative of common ancestry. Did this ancestor have "hystricomorph characteristics" or, as Wood (1950) has concluded, was the most recent ancestor shared in common a primitive rodent which had not yet developed these characteristics (Lower Eocene paramyid, perhaps)? If the modern African porcupine had greater serological similarity to New World hystricomorphs than it did to non-hystricomorph rodents (which probably had paramyid ancestry also) that fact might aid in answering the question. Unfortunately such evidence as we have is conflicting and inconclusive. One of our anti-*Hystrix* sera indicated greater serological similarity between the African porcupine and some American hystrico-

morphs than between the African porcupine and such a non-hystricomorph as the albino rat (table 2). Significant difference of this kind was not indicated by the other anti-*Hystrix* serum, however. Similarly, one anti-*Erethizon* serum indicated that the African porcupine is more closely related to the American porcupine than the albino rat is, but the difference exhibited by the other anti-*Erethizon* serum is probably not significant (table 1). Hence conclusions as to whether or not Old World and New World "hystricomorphs" have greater serological similarity to each other than they have to other rodents (e.g., Myomorpha) must await further investigation.

Do our results afford evidence that the North American porcupine is more similar to the South American hystricomorphs than it is to such non-hystricomorph rodents as the albino rat? Anti-*Erethizon* serum II (table 1) does not afford evidence of this, while anitserum III does indicate some degree of "serological unity" existing among the New World forms investigated. The results obtained with antiserum III agree with those of previous investigations in this laboratory. Finlay (1953) employed an anti-*Erethizon* serum which reacted somewhat more strongly with guinea pig and agouti sera (34% and 25%, respectively) than did the antisera used in the present investigation; its reaction with albino rat serum was only 4 per cent of the homologous reaction. Other studies offer evidence from the standpoint of reciprocal testing. Thus Lynch (1953) employed two anti-guinea-pig sera. Both indicated that the American porcupine is almost as similar to the guinea pig serologically as is the agouti (11%–20% vs. 17%–25%, respectively), a finding somewhat at variance with that of the present investigation. Both antisera had significantly weaker reactions with woodchuck and albino rat sera. Watson (1950) used an anti-agouti serum that had a 6 per cent reaction with American porcupine serum and an 18 per cent reaction with guinea pig serum. This antiserum did not react with albino rat and golden hamster sera, though oddly enough it did react weakly (ca. 2%) with the non-rodent sera: beef, domestic rabbit, whale and horse; it did not react with human serum. Thus despite vagaries of reaction of different antisera, all but one of the antisera used in this laboratory have given results tending to support the idea that the American hystricomorphs have greater serological similarity to each other than they have to non-hystricomorph rodents. But the differences between North and South American forms far outweigh the similarities; no heterologous reaction approaches even 50 per cent of the homologous reaction.

Systematists regularly place North American and South American hystricomorphs in the same taxon, at least at the subordinal level. Thus Wood (1955) included them both in Suborder Caviomorpha, but at the same time noted the fact, now clearly emphasized by our results, that the erethizontids are a taxonomically isolated group. This isolation is reflected in certain of their external parasites also (Vanzolini and Guimarães, 1955). Should the erethizontids be accorded their own suborder?

In this connection a study of the serological relationships of South American porcupines would be of interest. The three recent genera, *Coendou, Echinoprocta* and *Chaetomys,* are classed with their North American relative in Family Erethizontidae (Simpson, 1945). The first two named are included in the same subfamily with *Erethizon,* while *Chaetomys* is placed in its own subfamily. This arrangement suggests differing degrees of diversity within a narrow range. It would be interesting to know how closely degrees of serological similarity mirror conclusions based on morphology, and particularly to determine how much serological uniformity remains among these modern representatives of a family which dates back to the Oligocene. Are some or all of these South American por-

cupines more similar to each other serologically than they are to the migrant to North America? And are they more similar to North American porcupines than they are to non-porcupine rodents of South America? Or is the reverse true? Are they more similar to other South American hystricomorphs than the North American porcupine is? Answers to these questions would enable us to fit the South American porcupines into our diagram of relationships (fig. 3) and might shed light on the question of whether most of the serological divergence exhibited by the North American porcupine arose before or after its ancestors left South America.

Summary

Two anti-American-porcupine sera and two anti-African-porcupine sera were produced in male domestic fowl. The Libby Photronreflectometer was employed to measure the reaction of these antisera with sera from the following rodents: North American porcupine, African porcupine, guinea pig, agouti, albino rat, woodchuck.

The tests indicated that the New World and Old World porcupines have but slight serological similarity. This similarity is of about the same order of magnitude as that shown by either porcupine to the guinea pig and the agouti. Thus three sharply differentiated groups of hystricomorphs were demonstrated: (1) Old World porcupine, *Hystrix*, (2) North American porcupine, *Erethizon*, (3) South American hystricomorphs that are not porcupines, e.g., guinea pig and agouti. Each of these three has but slight serological similarity to the other two. Hence our findings correlate well with the fact that the Erethizontoidea were differentiated from other New World hystricomorphs as long ago as the Oligocene. And our results also support the view that the Old World and New World porcupines are but distantly related rodents that have developed many morphological resemblances to each other through parallel evolution on their respective continents.

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THE ORIGIN AND EVOLUTION OF THE VENOMOUS SNAKES

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In the virtual absence of a fossil record, the origin and subsequent evolution of the venomous snakes must be inferred from our knowledge ot their modern representatives. Evidence pertaining to this history has been accumulating since the days of Boulenger and Cope. However, the several interpretations of this evidence have led to conflicting phylogenetic schemes. These difficulties are to be expected from the limitations of the comparative method and the high degree of specialization by these snakes. The chief point of disagreement among the current and choice hypotheses is in the relationship between the Elapidae and the Viperidae. These families have long been regarded as independent derivatives of colubrid ancestry. The present paper is to introduce new evidence as to the relationships between the major groups of venomous snakes from a study of their vertebral characteristics. The results of this study, together with the evidence from other sources, indicate that the elapine and viperine stocks are intimately related. Enough evidence is available to suggest a course of the development of these families which will accommodate those features that have previously been regarded as ambiguous and inconsistent.

In order to reconstruct a phylogeny such as this, we seek those morphological attributes that have remained relatively stable in time. Vertebral form among snakes exhibits such stability. In a recent study the writer has explored the adaptive significance of vertebral form in snakes (Johnson, 1954). Vertebral shape was expressed in twelve measures and these in turn were combined into ratios to provide the characters for quantitative analysis. Only those ratios that showed low intra-columnar variability were employed. With a sample of 57 snakes representing eight families, the association of vertebral variety with mode of life was tested. The results of this study indicated that vertebral form is remarkably stable. Little association of the characters with various modes of life was found. Further, an attempt was made to demonstrate the association of the characters with mode of life in one family, the Colubridae. The same results were obtained; the colubrids showed little divergence with respect to gross adaptation.

An analysis of six families revealed that vertebral form reflected taxonomic relationships to a greater degree than mode of life. These results suggest that the variation of form expresses those primary adaptive tendencies correlated with the divergence of groups now recognized as taxonomic entities.

This study provided an assessment of the reliability of snake vertebrae as a source of phylogenetic inference. With such a background the vertebral characteristics of the following families were studied:

1. Colubridae: The largest of the families; this group consists chiefly of non-venomous snakes.
2. Elapidae: This family, comprising the cobras, kraits, coral snakes and their allies, are front fanged venomous snakes with relatively immobile maxillae.
3. Viperidae: The vipers are front fanged snakes possessing movable maxillae that allow the folding back of the fang at the close of the gape.
4. Crotalidae: The rattlesnakes and their allies are essentially vipers but

are distinguishable externally by their possession of the pit organ.

The poorly known rear-fanged Boiginae were not included in this investigation since it is quite clear that this sub-family of the Colubridae must be regarded as a phylogenetically heterogeneous assemblage. The sea snakes, Hydrophidae, are not included in the tests because of the small sample available to the author.

Materials and Methods

The majority of specimens used were obtained from the Chicago Natural History Museum (C.N.H.M.). Australian material was supplied by Mr. Wendell Swanson (W.S.), and a few specimens were collected by the author.

The following snakes were used in the analysis:

Colubridae
- *Abastor erythrogrammus* CNHM 38114
- *Atractus nicefori* CNHM 54881
- *Boiga irregularis* WS 17
- *Coluber taeniatus ruthveni* RJ
- *Dendrophis punctulatas* WS 18
- *Geophis chalybaeus* CNHM 20354
- *Imantodes cenchoa leucomelas* CNHM 70747
- *Lampropeltis getulus holbrooki* RJ 50
- *Liodytes alleni* CNHM 25944
- *Natrix erythrogaster transversus* CNHM 46325
- *Natrix rhombifera* CNHM 46307
- *Natrix sipedon confluens* CNHM 46300
- *Natrix tasselatus* CNHM 20893
- *Opisthotropis kuatunensis* CNHM 24869
- *Oxybelis fulgidus* CNHM 22376
- *Passerita prasinus* CNHM 71633
- *Thamnophis eques* RJ 15
- *Thamnophis sauritus proximus* RJ

Elapidae
- *Acanthophis antarcticus* WS
- *Boulengerina annulata annulata* CNHM 71836
- *Dendroaspis viridis* CNHM 22595
- *Maticora philippinus* CNHM 68918
- *Micrurus fulvius fulvius* CNHM 39170
- *Micrurus spixi obscura* CNHM 64728
- *Naja naja naja* CNHM 26842
- *Notechis scutatus* RJ 51
- *Pseudechis porphyriacus* RJ 52
- *Walterinnesia aegyptia* CNHM 72321

Viperidae
- *Atheris nitschei* CNHM 8987
- *Atractaspis microlepidota* CNHM 62104
- *Bitis gabonica* CNHM 22254
- *Aspis cerastes* CNHM 69252
- *Aspis vipera* CNHM 25320
- *Echis carinatus* CNHM 65918
- *Vipera aspis* CNHM 23438
- *Vipera berus* CNHM 21563

Crotalidae
- *Ancistrodon contortrix* RJ 18
- *Ancistrodon piscivorus* CNHM 37547
- *Crotalus atrox* RJ 12 and RJ 19
- *Crotalus cerastes* CNHM 26163
- *Crotalus horridus* CNHM 45092
- *Trimeresurus schlegelii* CNHM 2524
- *Trimeresurus wagleri* CNHM 71624

A deliberate attempt was made to make this sample as heterogeneous as possible with regard to habitus. Among the four families, there are twenty terrestrial animals represented, including swift runners, desert sidewinders, semi-aquatic and semi-fossorial forms. Eight aquatic, nine arboreal and seven fossorial species are present in the sample.

As in the previous study the basic data were obtained from radiographs. A description of the special X-ray technique employed has been presented elsewhere (Johnson, 1955). Measurements were made on a light box equipped with a vernier scale reading to tenths of a millimeter. The repeatability of measurement was found to be within 1 to 2 per cent. The following seven measures were found to be readily available from the radiographs and from previous experience to be biologically reasonable.

w.P.—greatest width across the prezygapophyses
w.Pt.—greatest width across the postzygapophyses
w.B.—least width of body
w.N.C.—least width of the neural canal
w.Ba.—greatest width of the condyle
P-Pt.—greatest distance between the pre- and post-zygapophyses
l.C.—length of the centrum along the midline, from the deepest point within the glenoid cavity to the posterior edge of the condyle.

Ten thoracic vertebrae starting with the fifth were measured at equal intervals in each column. These seven measures were combined into twelve ratios to minimize the effect of actual size and to reduce the X-ray distortion that could not be removed by the X-ray technique itself.[1] These ratios are:

#1. w.P./w.Pt.	#7. P-Pt./l.C.
#2. w.P./w.B.	#8. l.C./w.B.
#3. w.P./P-Pt.	#9. w.B./w.N.C.
#4. w.Pt./w.B.	#10. w.P./l.C.
#5. w.Pt./w.Ba.	#11. w.Pt./l.C.
#6. w.Pt./P-Pt.	#12. P-Pt./w.B.

These twelve ratios were previously found to be biologically meaningful and to exhibit minimum intra-columnar variance. The average value for each of the ratios, for each individual in the sample, comprises the raw data of the actual statistical tests used.

The Analysis

The analytical model was organized as follows:

I. Consider vertebral characters #1 to #12 and the families Colubridae, Elapidae, Viperidae and Crotalidae. To distinguish between families by the quantitative expression of characters #1 to #12.
II. Assume each taxonomic category to be as heterogeneous with respect to mode-of-life as any other taxonomic group.
III. For each character, test the null hypothesis that the taxonomic samples were drawn from the same population.

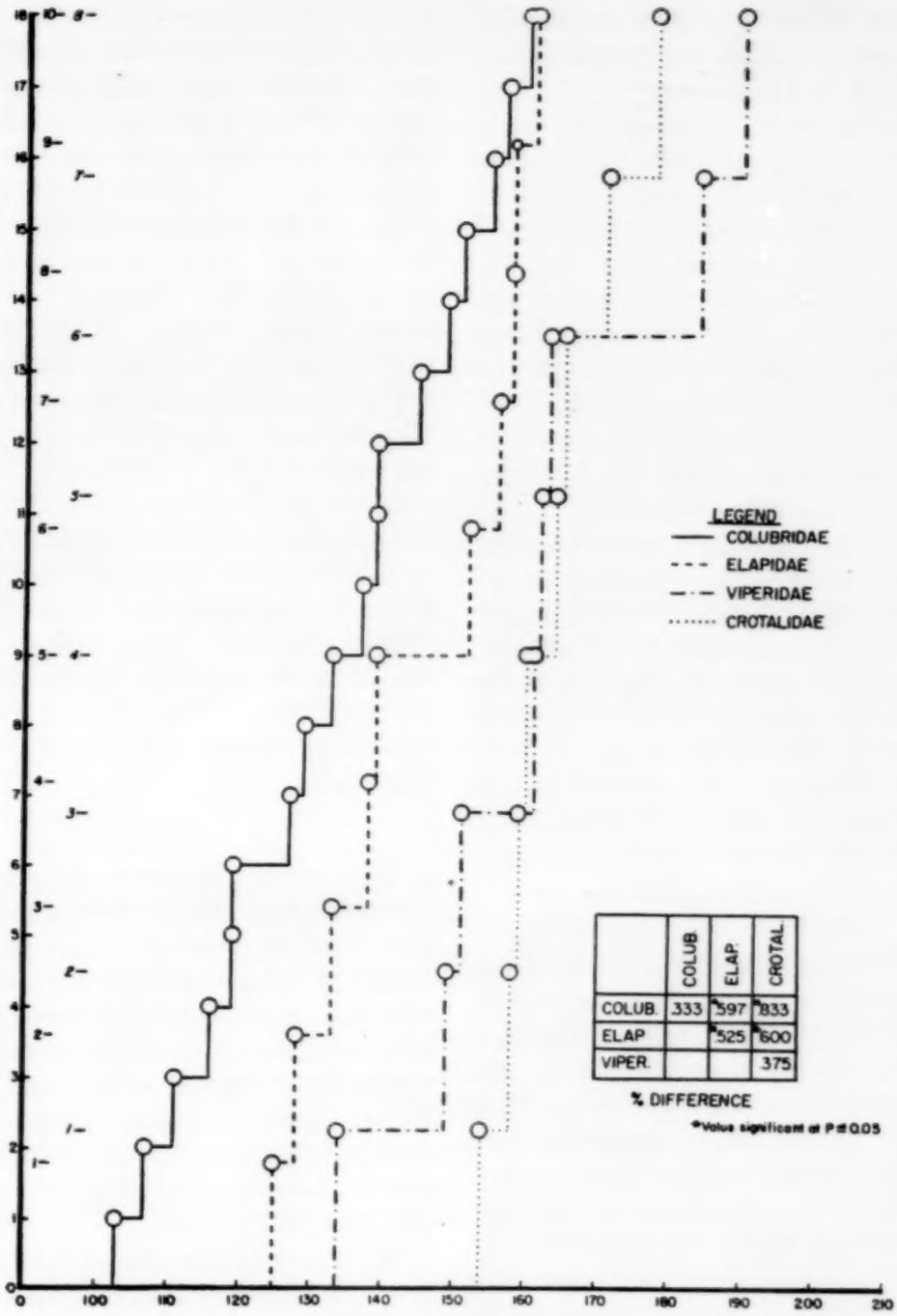
The non-parametric Kolmogorov-Smirnoff test of this hypothesis was used. This test enables us to compare the entire frequency distribution of a ratio within a given family with its distribution in the other families. The test thereby avoids the less meaningful comparison of family averages and the restrictive assumptions of most of the standard statistical procedures.

Seven of the characters employed were found to show statistically significant difference between two or more families (table 1). The cumulative frequency distribution of one of these characters is exhibited in figure 1. The differences detected by the tests are statistically significant group differences and no group was found that could be delimited by an exclusive range of character expression. The differences detected by the tests may be translated back into the differences in vertebral configuration by reference to the radiographs and cleaned skeletons. The results may be summarized as follows.

TABLE 1. *The characters found to be significantly different in the Kolmogorov-Smirnoff tests*

	Elapidae	Viperidae	Crotalidae
Colubridae	w.B./w.N.C.	w.Pt./l.C.	w.P./w.Pt. w.P./w.B. w.Pt./P-Pt. l.C./w.B. w.B./w.N.C. w.Pt./l.C.
Elapidae	———	w.P./w.Pt. P-Pt./l.C.	w.P./w.Pt. w.P./w.B. w.Pt./l.C.
Viperidae	———	———	———

[1] The rationale of the sampling and the entire analysis of such data has been discussed in full in the previous work alluded to throughout the text (Johnson, 1955).



	COLUB	ELAP	CROTAL
COLUB	333	*597	*833
ELAP		*525	*600
VIPER			375

Figure 1. THE CUMULATIVE FREQUENCY DIAGRAM FOR RATIO w.Pt./l.C.

Elapidae

The elapids agree more closely with the Colubridae than any other group. The single difference found between these two families appears to be minor, and arises from a tendency of the elapids towards a narrower neural canal relative to vertebral body width. This tendency is by no means consistently expressed throughout the family.

Two instances of difference were found between the elapids and vipers. The elapids have higher values for the character w.P./w.Pt. and lower values for P-Pt./1.C. than do the vipers. Both of these tendencies can be traced to a single feature. The elapid prezygapophyses are more often at right angles to the axis of the vertebrae, while in the vipers this structure is directed more forward. The

effect on the ratios is to give the elapids smaller values for P-Pt. and larger values for w.P. than in the vipers.

The elapids were found to differ in three characters with the Crotalidae: w.P./w.Pt., w.P./w.B. and w.Pt./l.C. The dissimilarity between elapids and crotalids is more striking than the differences seen between the elapids and the other families. The prezygapophyses of the elapids exceed the postzygapophyses while among the crotalids these structures are more often equal in width. The vertebral body among the Elapidae is narrower and more elongate.

In summary, the elapid vertebrae may be described as bearing prominent prezygapophyses usually well exceeding the postzygapophyses in length and arising at nearly a right angle to the axis of the vertebral body. The vertebral body tends to be quite narrow and elongate. In total aspect the vertebral form resembles that of swift terrestrial snakes as exemplified by the colubrid genus Coluber.

Viperidae

The vipers were found to differ from the Colubridae in one instance, w.Pt./l.C. (fig. 1). The higher values obtained among the vipers were traced to the viperine tendency toward relatively short vertebrae. The results of the comparison of vipers and elapids are discussed earlier. No differences were found to be significant between vipers and crotalids.

The viperid vertebrae are relatively short and broad in body. The zygapophyses are nearly equal in length and the prezygapophyses are distinctly directed forward. The general aspect is one of stockiness. Among the larger forms this feature gives the vertebrae a massive appearance reminiscent, in this respect, of the Boidae.

Crotalidae

The crotalids differed from the Colubridae in six instances. Crotaline snakes have lower values for w.P./w.Pt. as their zygapophyses are more nearly equal in width. The lower values for w.P./w.B. among the crotalids represents a relatively smaller expression of the prezygapophyses and a greater vertebral width. The stocky tendency of the crotalids was expressed by relatively lower values for P-Pt., l.C. and higher values for w.B. than in colubrids. This characteristic appears in the differences obtained for the characters w.Pt./P-Pt., l.C./w.B., w.B./w.N.C. and w.Pt./l.C. The results of the comparisons made between crotalids, elapids and viperids were discussed earlier.

The vertebrae of the crotalids are identical in form to those of the Viperidae. The differences found between the Crotalidae, Elapidae and Colubridae not shared by the vipers arise from the emphasis of viperine tendencies among the crotalids. The crotalid vertebra is relatively shorter and broader than the viperid.

Conclusions

In general the tests do not indicate great differences in vertebral form among the families studied. No group was found to be as readily definable as, for example, the Boidae on these characteristics alone. This observation suggests again the temporal stability of vertebral form. It is possible to recognize, on the basis of vertebral shape, two groups of related families: the elapid-colubrids and the viperid-crotalids. The dissimilarity between colubrids and crotalids is far more striking than that between the elapids and crotalids.

The statistical analysis has functioned in this study to magnify and draw attention to structural deviations at a high taxonomic level that might otherwise have been obscured by an overall morphological similarity. Alone, the findings of this exploration are inconclusive with respect to the relationships between these families. However, in the context of other data from other sources, the vertebral evidence renders some hypotheses less probable than others. Before pre-

senting such a demonstration, it is necessary to review this additional evidence. Morphological data pertinent to the history of venomous snakes is available from studies on the skull, dentition, venom, venom glands, scutellation, eyes, jaw muscles and trunk musculature.

Skull and Dentition

The skull of the elapids is nearly identical with the colubrid skull and may be distinguished by the anterior fang and the reduction of teeth on the maxilla. At first glance the viperid skull is in sharp contrast but there are basic similarities. The distinctiveness of the viperid skull can be traced to the extreme kineticism developed in it, to provide the fang erection mechanism. The fang bearing maxilla is hinged to the prefrontal and can be rotated forward as the quadrate is forced down, pushing the pterygoid and ectopterygoid forward and against the base of the maxilla. The viperine skull gives the impression of being shortened anteriorly and posteriorly so that the quadrates and pterygoids appear to be extended farther back than in the typical colubrid. The resulting aspect is one of fragile complexity when compared to the heavier and compact appearance of the elapid skull. The elapids also exhibit kineticism to a limited degree (Haas, 1930). The Australian elapid *Acanthophis* is reported to approach nearly the viperine condition in fang mobility (Kellaway, 1933). Skulls of vipers and pit vipers are nearly indistinguishable.

The elapid fangs are borne upon the anterior end of a solid maxilla and are commonly followed by a series of small teeth. The dentition of cobras has been thoroughly reviewed by Bogert (Bogert, 1943). The viperid and crotalid maxilla is quite specialized and serves as a bony base for a single tooth, the fang. The closure of the fang groove is more perfect in the vipers and crotalids than in the elapids. In length the fangs of most vipers and pit vipers greatly exceed those of the elapids.

Venom and Venom Glands

Until fairly recently it was believed that the elapine and viperine snakes differed in the physiological action of their venoms. It was held that the elapids are consistent in possessing a venom which is "neurotoxic" in action. In contrast, the viperine venoms were characterized by their haemolytic properties. Further studies have indicated that no such distinction can be made. Kellaway found that the Australian elapids have venoms in which both neurotoxic and haemolytic components are evident (Kellaway, 1933). He observed that the Australian elapids differed from Oriental members of this family in many properties of their venom. Klauber (1936) states that snakes of the same genus may possess venoms of either physiological type. Among the opisthoglyphs both venom types are represented (Smith and Bellairs, 1944). It is clear that venom is not a trustworthy taxonomic criterion at the level we are concerned with here.

The venom glands of the Elapidae, Viperidae and the Crotalidae are similar in structure. There are no indications as to how these glands evolved for no intermediate conditions are known among these families or among aglyphous colubrids. Quite possibly the venom glands evolved from a differentiation of the anterior end of the superior labial gland (Smith and Bellairs, 1947). The little embryological detail known indicates that the venom and the labial glands arise independently from separate epithelial buds (Martin, 1899). The venom of the rear fanged snakes is a product of the parotid gland, shown clearly by Smith and Bellairs to be a differentiation of the posterior portion of the superior labial.

Head Shields

There exist no constant differences between the elapids and viperids with regard to the condition of the head shields. There is a tendency in the vipers and crotalids to break up the large dorsal

shields into many smaller elements but such forms as *Azemiops, Causus, Atractaspis* and *Ancistrodon* show the typical colubrid and elapid pattern.

Eyes

From a study of the remarkable eye anatomy of the snakes, Wall (1942) found that the retinae of elapids, vipers and crotalids are all readily derivable from the diurnal colubrid pattern. The eye of the elapids is anatomically closer to that of the colubrids than are the eyes of vipers and crotalids. *Acanthophis,* however, has a viperine retina. Elapids possess round pupils while those of the vipers and crotalids are slit like. A few primitive viperid genera, *Causus* and *Atractaspis,* are round pupilled.

Jaw Musculature

In a series of papers Haas has studied the condition of the jaw musculature in many snake families. The general evolutionary direction of jaw musculature apparently has been toward simplification. Haas has concluded that the complicated arrangement characteristic of the viperids and crotalids could only have been derived from some very primitive colubrid. The comparatively simple jaw musculature of the elapids can be readily derived from the simple (more advanced) colubrid condition (Haas, 1938).

Trunk Musculature

Mosauer (1935) studied the myology of the trunk region of an extensive series of snakes. The results of these studies, as they pertain to the present problem, may be summarized as follows. The trunk muscles of snakes are quite constant in their arrangements and are not subject to last moment specialization. Among colubrids of quite varied modes of life, the same muscle pattern was evident. Mosauer recognized three major myological types; boid colubrid and viperid. The elapid musculature was found to be identical with the colubrid type. That of the vipers and crotalids resembles the Colubridae in general but shows certain constant differences. He concluded that the vipers are more primitive in some respects than the present day Colubridae and suggested that these groups were derived from a common ancestral stock. Interestingly enough, the elapid *Acanthophis* and the cobras are of the viperid musculature type.

Vertebrae

In addition to the vertebral characteristics already mentioned, elapids, viperids and crotalids all possess hypapophyses developed throughout the vertebral column. The more common condition is for these structures to be restricted to only the more anterior and the most posterior thoracic vertebrae.

Discussion

The number of phylogenetic relationships possible with four taxons is large but the evidence reviewed above suggests that only a few possibilities need be taken seriously. Three theories deserve attention. Boulenger (1893) proposed that the viperids and crotalids are of opisthoglyph ancestry, while not differentiating between the elapids and the Colubridae. Other students have proposed that the viperids arose from primitive aglyphous colubrid stock and the elapids from a far more advanced (and later) colubrid. A few have suggested that the elapids and viperids are closely related. Bogert suggests further that the vipers arose from primitive elapids (Bogert, 1943). All appear to agree that the elapid and viper lines are colubrid or colubrid-like in ancestry. Also it seems clear that the Hydrophidae are sea going elapids and the crotalids are of viperine stock.

The arguments offered in support of an opisthoglyph (Boiginae) ancestry for the viperine snakes are based primarily upon a resemblance of venoms and the fang position on the maxilla. It is now clear that the opistoglyph and viperine

venoms are not unique. The physiological properties of venoms are not reliable sources of phylogenetic inference for among all groups of venomous snakes venoms of both physiological types are represented. It has been argued that the fang of the vipers is borne upon the posterior end of the maxilla and therefore agrees in position with the fangs of opistoglyphs. This writer can see no conclusive way of determining from the highly specialized and reduced viperine maxilla on which end of some ancestral maxilla the present fang is borne. There are several genera of opisthoglyph snakes in which the maxillary series is reduced, but the rear fang remains a rear fang. The simplest interpretation of the viperine condition is to regard the position of the fang, with respect to the rest of the skull, as primary.

The evidence available suggests that the venom glands of vipers and opisthoglyphs are not homologous organs (Smith and Bellairs, 1947). There are no other features of this heterogeneous group that particularly qualifys them for a place in viperine history and there appear to be some that disqualify them. The issue is resolved, then, to whether the vipers and elapids represent independent or related stocks derived from aglyphous colubrids.

The arguments for the independence of elapids and vipers are based upon differences in the skull, dentition, eyes, jaw and trunk musculature. Could the permeating influence of extreme kineticism be subtracted from the viperine skull, the skulls of elapids and vipers would certainly agree more closely than do many of the colubrids among themselves. The degree of kineticism attained in vipers can be regarded as a continuation of a trend in this direction already evident among elapids (Bogert, 1943). The viperid maxilla is readily obtained by a continuation of the reduction of the present elapid maxilla. This reduction would seem far less drastic than that proposed for an opisthoglyph ancestry where it was necessary to have all the anterior teeth removed and the rear fang along with the posterior end of the maxilla brought forward. The difference in retinae between the elapidae and vipers is bridged by the elapid *Acanthophis* with its viperid eye. The round pupil of the elapids is found among primitive viperid genera. The differences in trunk musculature are compromised by the viperid arrangement of the cobras and *Acanthophis*.

The resemblance of the simple jaw musculature of the elapids to advanced colubrids and that between the complex jaw musculature of the viperids and primitive colubrids, is the only feature that appears to consistently support the theory of independence. If we accept these resemblances of jaw musculature patterns as reflecting a common phylogeny, then it is necessary to regard the complex alkaloid toxins and the front fangs of these snakes as points of convergence. The cobras would have to be considered as regressing in their trunk musculature towards the primitive colubrid or viperine condition. The position of *Acanthophis* would seem even more embarrassing.

Throughout his extensive studies Haas has found close correspondence of muscle patterns with taxons of the familial and generic rank. This is in sharp contrast to the lack of such definitiveness in the trunk musculature as reported by Mosauer. Mosauer, in a study that included fifteen families, found that all variations could be placed in three categories. Further, "Each of these main types is to be distinguished not by one character only but by several correlated peculiarities of muscle arrangement, which are, indeed, surprisingly constant" (Mosauer, 1935). It would appear that the trunk musculature has in general remained more stable in time than has jaw musculature. Haas found many differences in the jaw musculature of two closely related species of the genus *Causus* (Haas, 1938). If jaw musculature is subject to relatively late specialization among snakes, then it is not inconceivable that in the great time

available since the origin of these groups, that convergences could also occur. The resemblance, in jaw musculature, of the Boidae and the Colubridae must surely be such a convergence since Haas has found highly complex arrangements among primitive colubrid genera. It is much simpler to regard the resemblance of the jaw musculature between advanced colubrids and elapids as convergence, rather than to accommodate the many inconsistencies of a hypothesis to the contrary.

The vertebral evidence from the present study does not favor the hypothesis that the elapids and viperids are of independent colubrid ancestry. If the vipers are of primitive colubrid stock and the elapids derived from advanced colubrids, then the differences between the viperid derived crotalids and the elapids should be at least as striking as those between the crotalids and colubrids. The results of the tests performed here indicate that this is not the case. The apparent stability of vertebral form lessens the probability of resemblance without a common phylogeny. If we accept the measures employed as truly representative of vertebral form, then the differences found between these groups are best accounted for by postulating that the elapids lead from a basal colubrid stock to the vipers and the vipers to the crotalids. The arrangement is precisely that suggested by Bogert (1943).

It seems likely that the elapids and viperids arose from a common stock. Not only do they agree in many features but there exist morphological links that bridge their differences. Since the elapids have retained more colubrid characteristics than the viperids, they must more closely approximate this basal stock today. With such an interpretation, the retention of small maxillary teeth and the incomplete closure of the fang groove can be regarded as primitive characters among the elapids. The remarkable racer-like appearance of the vertebrae of many elapids suggests that swiftness has been involved in their early radiation. The common vertebral characteristics of swift moving snakes are one of the few exceptions to the general stability of vertebral form with respect to mode of life. The differences in body musculature now seen between the elapids and the powerful, but slower moving, viperine snakes may be attributable to this early divergence in locomotion.

The viperine snakes, arising from some elapid, possibly resembling *Acanthophis*, have concentrated upon quantity in venom rather than higher potency as developed among the elapids. This is implied by the modifications of the viperine skull to accommodate a larger gland as well as fangs. For the viper to place more venom deeper in the prey, the powerful body, with its peculiarities of trunk musculature, must have been involved in this specialization. With no extraordinary demands upon their jaw musculature the elapids appear to have joined the general group tendency to reduce and simplify. The viperids, on the other hand, utilized the complex primitive pattern to accommodate the extrusion of greater quantities of venom and the accompanying modification in skull kineticism. The crotalids may be regarded as the latest models in the viperine line with such useful accessories as a sensory pit capable of detecting warm blooded prey in the absence of light.

The Elapidae include some rather remarkable deviations from their otherwise consistent form. The cobras and *Acanthophis* have several viperine characteristics. The genus *Bungarus* is unique among snakes for its complete lack of iris pigmentation and among the elapids for the weird development of winged prezygapophyses. The Australian forms differ from the rest in the composition of their venom. Such features of the group have prompted the suggestion that the elapids are polyphyletic in origin. There is, however, no conclusive evidence yet available to support such a contention.

If this phylogenetic interpretation of the venomous snakes is correct, than the

elapids represent an older radiation than do the Colubridae. This supposition is supported by the scant paleontological data available. It is possible that venom and the venom apparatus were developed when competition for food was intense. As this condition was alleviated, the non-venomous colubrids had the opportunity to undergo their major radiation without the advantage of venom. Unless some such postulate of lessening competition between venomous and non-venomous snakes is made, it seems difficult to understand why the venomous snakes have not completely dominated our present herpetofauna. That their advantage is great enough is evident by the very existence of the venomous families and the many instances of slightly or appreciably venomous colubrids. As interesting as these groups are to the student of evolution, mankind would certainly find the rest of the earth less comfortable had it the snake fauna of modern Australia.

Summary

The present paper introduces new evidence as to the relationships between the major groups of venomous snakes from a study of their vertebral characteristics. Vertebral form was expressed in seven measures and combined into ratios to provide the characters for quantitative analysis. A sample of 44 snakes, representing the families Colubridae, Elapidae, Viperidae, and Crotalidae, was subjected to an analytical model employing the non-parametric Kolmogorov-Smirnoff test. The results of the analysis indicated that the vertebrae of elapine, colubrine and viperine snakes are quite similar in form. The differences between colubrids and crotalids were found to be far more striking than those between the elapids and crotalids. In the context of morphological data from other sources, it was concluded that the differences between these families are best accounted for by postulating that the viperine and crotaline snakes were derived from a primitive elapid stock.

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A FURTHER REPORT ON THE GENETICS OF THE MACHAON GROUP OF SWALLOWTAIL BUTTERFLIES

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In previous papers (Clarke and Sheppard, 1953, 1955) we described hybrids obtained in captivity between various species of the *machaon* group of swallowtails; we also gave preliminary information about some of the genetic differences between the butterflies concerned.

The work was continued during 1954 and although it was greatly hampered by bacterial disease, which affected the larvae of almost all broods, new hybrids and data were obtained and these are discussed in the present paper.

THE INHERITANCE OF LARVAL SPOT COLOUR IN *P. machaon* AND *P.p. asterius*

Previously (Clarke and Sheppard, 1955) data had been obtained which suggested that the difference between the orange or red spots on the larva of *P. machaon* L. and the yellow ones found on the larvae of many *P. polyxenes asterius* Cram. and *P. brevicauda* Saunders is controlled by a pair of allelomorphs and that yellow is recessive to orange. The colour was scored by comparison with the colours in the colour atlas of Villalobos and Villalobos (1947) and was given numerically by the number of the page on which the matching colour was found. New data on the inheritance of larval spot colour are given in table 1. The new information confirms the hypothesis that the difference between orange and yellow is due to a pair of allelomorphs. It will be seen from the table that there is a clear cut difference between orange and yellow, no spot colour having fallen on page 9 of the atlas.

Evidence is accumulating that there are other genes affecting this character. Some of the heterozygotes for orange, in the backcross broods, have a colour score of 8 whereas in the F_1 hybrids, which are also heterozygotes, the colour seldom has a score greater than 6 except when the *P. machaon* parent is of the subspecies *hippocrates* Felder (see table 2 brood 41B). These F_1 hybrids between *P.m. hippocrates* and *P.p. asterius* showed considerable variation in respect of the colour of the orange spots on the larvae. There was a tendency for them to be more yellow than those found in F_1 hybrids between *P.p. asterius* and *P.m. britannicus* Spengel or *P.m. melitensis* Ellen from Malta, or even the orange spotted groups of larvae from hybrids backcrossed to *P.p. asterius* (tables 1 and 2). This suggests that the allelomorph at the locus controlling the presence of orange spots in *P.m. hippocrates* may be different from the one in *P.m. britannicus*. Alternatively, the Japanese subspecies may carry modifiers affecting the dominance of the gene controlling spot-colour. However, in view of the abnormal sex ratio obtained among hybrids of this subspecies and the fact that the chromosome number may be different from that of *P. machaon*, and perhaps other members of the group, much more genetic and cytological work must be undertaken before any valid interpretation of the apparent difference in dominance between different hybrids can be made.

TABLE 1

The table shows segregation for larval spot colour in two backcrosses. The colour of the spots is indicated by the number of the page of the colour atlas on which a corresponding colour was found (see text). 4 and 5 are red, higher numbers have increasing amounts of yellow present and a decrease in the amount of red, 10 and 11 being pure yellow.

Brood No.	Back-cross matings	Orange (pages 5, 6 and 7)	Pale orange (page 8)	Yellow orange (page 9)	Yellow (pages 10 and 11)	Total
61	♀ *P. p. ast.* × ♂ hybrid (♀ *P. p. ast.* × ♂ *P. m. medit.*)	6 (4 on page 5, 2 on page 6)	0	0	4 (all on page 10)	10
118	♀ *P. p. ast.* × ♂ hybrid (♀ *P. mach. brit.* × ♂ *ast.*)	18 (5 on page 6, 13 on page 7)	12	0	22 (17 on page 10, 5 on page 11)	52

SEX RATIO IN HYBRIDS OF *P. machaon hippocrates*

There is a departure from 1 : 1 sex ratio in brood 89 (table 3) ($\chi^2 = 5.6$ $P < 0.02$). Although the other F_1 and backcross families are too small taken by themselves all show the same deficiency of females that is apparent in brood 89 ($\chi^2 = 11.8$, $P < .001$). This is in marked contrast to the situation found in other hybrids of the *machaon* group so far investigated. In these other hybrids no great departure from a 1 : 1 sex ratio was obtained. Deficiency of females, the heterogametic sex in lepidoptera, is in agreement with predictions made by Haldane (1922). He suggested that if one of the sexes in hybrids tends to be rare, absent or infertile, it will usually be the heterogametic sex. In connection with the behaviour of the sex ratio in the *P.m. hippocrates* hybrids, it is of interest

TABLE 2

The table gives the colour of the larval spots in *P. m. hippocrates* hybrids. The species involved in these hybrids can be obtained by looking at the corresponding brood number in table 3.

Brood No.	Orange (pages 4, 5 and 6)	Pale orange (page 7 or 8)	Yellow (pages 10 and 11)	No. of larvae scored
41B	0	11 (5 on page 7, 6 on page 8)	0	11
50	8 (2 on page 4, 6 on page 5)	0	0	8
89	29 (2 on page 4, 26 on page 5, 1 on page 6)	0	0	29
175	14 (6 on page 5, 8 on page 6)	0	0	14

TABLE 3. *Sex ratio of the hybrids of which one parent was P. m. hippocrates or its hybrid is given in the table*

In all the broods a large number of individuals died as larvae, probably as the result of disease. In brood 41B where the ground colour of the butterfly was black, there was a difference in the amount of yellow in the males and females. This sexual dimorphism is similar to that found in *P. p. asterius* (see Clarke and Sheppard, 1955).

Brood No.	Mating	No. of pupae which did not emerge	Offspring ♂	Offspring ♀	Remarks
41B	*P. p. asterius* ♀ ×*P. m. hippocrates* ♂	4	4	1	Hybrid butterflies had a black ground colour. Sexual dimorphism present
50	*P. mach. medit.* ♀ ×*P. m. hippocrates* ♂	No record	2	0	
89	*P. m. hippocrates* ♀ ×*P. mach. brit.* ♂	10 died as pupae	22	8	Whole brood emerged at intervals over 3 months (from 8.5.54 to 5.8.54). ♀ ♀ very late
175	Backcross *P. mach. gorganus* ♀ × ♂ from brood 50	1	4	0	

to note that Maeki & Makino (1953) reported that this subspecies had a haploid chromosome number of 31, whereas Lorkovic (1941) reported a haploid number of 30 for a European subspecies of *P. machaon*. If these really have different chromosome numbers (which is uncertain, as Federley (1939) reported a chromosome number of 30 to 33 in the males), this difference may account for the deficiency of females. Brood 175 shows that at least some of the males are

TABLE 4

No butterflies were obtained from *P. hospiton* hybrids and many of the larvae died of disease even before they could be scored for spot colour or the pattern of black bands on the larvae.

Brood No.	Mating	Larval spot colour	Nature of black bands on larvae	Total larvae scored
181	*P. m. gorganus* ♀ ×*P. hospiton* ♂	orange page 5	slightly castellated	1
183	*P. p. asterius* ♀ ×*P. hospiton* ♂	yellow page 9	slightly castellated	9
195	*P. p. asterius* ♀ ×*P. hospiton* ♂ (same ♂ as 183)	6 larvae, none scorable	not scorable	0
232	*P. m. britannicus* ♀ ×*P. hospiton* ♂	7 larvae, none scorable	not scorable	0
234	*P. m. saharae* ♀ ×*P. hospiton* ♂	30 eggs laid, many hatched, all died before being scorable		0
240	*P. m. britannicus* ♀ ×*P. hospiton* ♂	2 larvae, not scorable	not scorable	0

fertile but as yet there is no information of the fertility of the females.

P. hospiton Hybrids

Larval spot colour: Unfortunately all the hybrids between *P. hospiton* Géné and other members of the *machaon* group died in the larval stage, almost certainly as the result of bacterial disease. Consequently little information on the inheritance of the colour of the orange spots on the larvae is available. The hybrid with *P. machaon* had orange spots, but unlike the hybrids between *P. machaon* and *P.p. asterius*, which have orange spots, those with *P.p. asterius* had spots almost as yellow as those of larvae of *P.p. asterius* itself (table 4). Until more specimens and genetic data are available no interpretation of this apparent almost complete reversal of dominance can be made; however, the phenomenon is clearly worth further study.

P. machaon saharae Hybrids

Eggs of *P. machaon saharae* Oberthür were obtained from Mr. Kenneth Guichard from Benghazi in May 1954. The full-grown larva of *saharae* is strikingly different from that of *machaon* (see fig. 1). The main differences are:

1) In *machaon* the transverse black band carrying the orange spots is wider than, or approximately the same width as, the intervening intersegmental black band. Both bands are straight.

In *saharae*, the black bands carrying the spots are narrow and do not entirely surround the orange spots. The intersegmental black bands are discontinuous, with a sharply castellated outline, the castellations being so prolonged that they often join up anteriorly and posteriorly with the next black band, giving the impression that there is a longitudinal dorsal black stripe.

2) In *machaon* the background is bright green, whereas in *saharae* it is

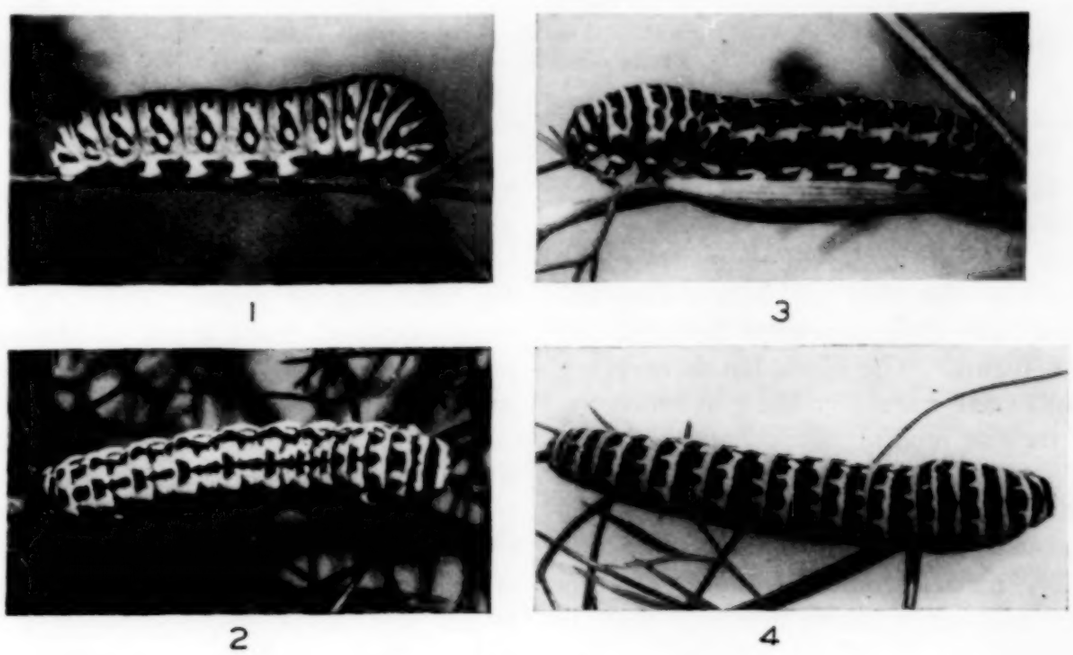

Figure 1.

(1) Larva of *P. machaon*, showing wide transverse black bands.
(2) Larva of *P. machaon saharae*, showing irregular interrupted black bands and antero-posterior stripe.
(3) Larva of hybrid *P. machaon* ♀ × *P. machaon saharae* ♂, showing intermediate appearance.
(4) Larva of *P. machaon* ♀ × *P. hospiton* ♂ (Corsica). Note irregularity of black bands with castellations and similarity to *P. machaon* × *P.m. saharae* hybrid larva.

TABLE 5

The table shows the results obtained from broods segregating for the colour patterns which distinguish the larvae of *P. machaon* from the subspecies *P. m. saharae*. One of the parents of brood 316 was from a larva which was hybrid-like, the other was from a larva which was *machaon*-like.

Brood No.	Mating	Total larvae	Description of larvae		Offspring	
			Machaon-like black bands, bright green background	Hybrid-like black bands, intermediate background	♂	♀
223	*P. m. brit.* ♀ ×*P. m. sah.* ♂	10	0	10	1	9
234	*P. m. sah.* ♀ ×*P. hos.* ♂	30 eggs laid, many hatched, all died before being scorable				
272	Backcross ♀ 223 ×*P. m. medit.* ♂	8	6	2	5 (1 died as pupa)	2
274	Backcross ♀ 223 ×*P. m. medit.* ♂	1	0	1		1
325	Second backcross ♀ 274 ×*P. m. medit.* ♂	21	7	14	2 rest died as larvae	2
316	Sib-mating between backcross hybrids ♀ 272×♂ 272	60 most died early, 3 only scorable	1	2	0	0

very light, almost white, and it is much less in evidence.

The F_1 hybrid larvae and those described in the text as hybrid-like are intermediate in appearance between the parent forms. The black bands carrying the spots are narrower than in *machaon*. The intersegmental black bands are irregular and castellated but not to the extent of *saharae*, so that there is not the appearance of a longitudinal black band. The green background is intermediate in colour and far more in evidence than in *saharae*.

In the backcross larvae (broods 272 and 274) and in the sib mating between backcross hybrids (brood 316) it will be seen that the larvae segregated cleanly as *machaon*-like and hybrid-like.

A second backcross generation was obtained (brood 325) and in this brood also the larvae segregated.

Four sib matings were obtained from the offspring of mating 223. No eggs were obtained from three of these, but from the fourth (brood 273) 15 eggs were obtained, of which three changed colour but did not hatch. As development was seen fertilisation must have taken place. This observation suggests that *saharae* has diverged from *machaon* sufficiently to show some reproductive isolation because in matings between individuals from the same population nearly all the eggs that show signs of development hatch. Moreover, if any of the eggs are fertile nearly all of them are fertile. Thus the F_2 hybrids between *P.m. britannicus* and *P.m. saharae* differ from the offspring of individuals showing

no reproductive isolation in two respects. Firstly only a small proportion of the eggs showed signs of development and secondly none of those that did start to develop hatched.

The data are inadequate for any final judgment, but the difference in the pattern of black markings in *P.m. saharae* larvae compared with other members of the *machaon* group appears to be due to a pair of allelomorphs showing no dominance, since the segregation in the backcrosses shows a good agreement with the expected 1 : 1 ratio.

Hybrid Larvae of *P. bairdi* and *P. machaon gorganus*

Four pupae of *P. bairdi* Edwards obtained from Arizona were kindly sent in January 1954 by the Reverend D. L. Bauer. Three of the pupae died but the remaining one produced a ♂ butterfly which was mated with a ♀ *P. machaon gorganus* Fruhstorfer. Four eggs were laid, all of these were fertile, but one was lost. The three larvae died when nearly full-grown. The larval spot colour was orange (page 5 of atlas) and the background bright leaf green.

The larva of *P. bairdi* has bright orange spots with a tendency to red and the ground colour is bright leaf green (Bauer, personal communication, and Bauer, 1955).

Discussion

In a previous paper (Clarke and Sheppard, 1955) evidence was presented which suggested that the difference between the yellow spots on the larvae of *P.p. asterius* and *P. brevicauda* on the one hand and the orange or red ones of *P. machaon* from Europe on the other is controlled by a pair of allelomorphs and that yellow is recessive or nearly so. New data confirms this interpretation (table 1). *P. hospiton*, a species confined to Corsica and Sardinia, also has a larva with red spots and hybrids between this species and *P. machaon* from Europe have red spots. However, the F_1 hybrid between *P.p. asterius* and *P. hospiton* has yellow spots. No interpretation of this apparent reversal in dominance can be made at the moment. However, the results do indicate that there is a genetic difference in the control of the red spots in *P. machaon* larvae as compared with those in *P. hospiton*.

The pattern of black marks on the larvae of *P. hospiton* resembles, in some respects, that found on *P.m. saharae* larvae rather than that on North American, Japanese or other European Swallowtails of the *machaon* group investigated by us, including those from Malta. The hybrids between *P. hospiton* on the one hand and *P. machaon* and *P.p. asterius* on the other have a pattern intermediate in appearance. Although no genetic data on the mode of inheritance of this pattern is yet available, the difference between the pattern of black in *P.m. saharae* and that in most other members of the *machaon* group has been investigated. The difference appears to be due to a pair of allelomorphs showing no dominance, the heterozygote being intermediate in appearance. The two members of the *machaon* group with the abnormal markings on the larvae are from Corsica and Sardinia and North Africa respectively. In this connection it is of interest to note that Eller (1936), partly on other evidence, has suggested that the presence of *P. machaon* and *P. hospiton* on the same islands is due to a double colonization and that the ancestors of *P. hospiton* came from North Africa, whereas those of *P. machaon* came from Europe. It is therefore of considerable importance to discover if the abnormal larval pattern is controlled by the same allelomorph in *P.m. saharae* and *P. hospiton*. It would also be of interest to know if the F_1 hybrids between *P.m. saharae* and *P.p. asterius* have larvae with orange spots as they do when other subspecies of *P. machaon* are used or whether they are yellow as is the case with hybrids of *P. hospiton* and *P.p. asterius*.

Mayr (1942) pointed out that for *P. hospiton* and *P. machaon* to be sympatric they must have diverged before meeting. It is therefore of considerable importance to note that a high frequency of abnormal development among the eggs of hybrids of subspecies of *P. machaon* inhabiting Europe has not been observed. These include hybrids between *P.m. britannicus* and *P.m. gorganus, P.m. britannicus* and *P.m. melitensis* from Malta, *P.m. britannicus* and *P.m. hispanicus* Eller from Spain as well as *P.m. gorganus* and *P.m. melitensis*. It is only the subspecies, *P.m. saharae,* from North Africa which has apparently diverged in such a way that abnormal development is found among the F_2 hybrids. Both morphological and genetic evidence, as far as it goes, support the view that *P. hospiton* is of North African origin and has evolved from a *P. machaon*-like ancestor, which was also the ancestor of *P.m. saharae*.

Another point of interest which has emerged from the present investigation is the abnormal sex ratio in the F_1 hybrids of *P.m. britannicus* and *P.m. hippocrates*. This may indicate that *P.m. hippocrates,* which unlike most other subspecies of *P. machaon* shows seasonal dimorphism in the female, may really be a distinct species. This view is strengthened by the fact that it has been reported to differ in chromosome number from a subspecies of *P. machaon* from Europe.

In view of these findings it is important to obtain hybrids between the N. American subspecies of *P. machaon* and the European and Asiatic forms and to investigate the chromosome numbers of the various races.

Most of the N. American members of the *machaon* group were apparently present in America and isolated from the Asiatic forms during the ice age. However, *P. machaon aliaska* Scudder, which is found in Alaska, appears not to be one of these but to be the result of an invasion from Asia after the ice age. In Manitoba there is another subspecies, *hudsonianus* Clark, which shows strong affinities with other members of the North American *machaon* group, especially *P. oregonia* Edwards. This is particularly clear in the spot on the anal angle of the hindwing. The black mark which forms a posterior border to the red area is broad and tends to be pear shaped as in *P. oregonia* and the anterior black border is much broader than in most subspecies of *P. machaon*. These two characters are controlled multifactorially (Clarke and Sheppard, 1955). An investigation of the chromosome numbers of the subspecies of *P. machaon* and the genetic differences between them might well reveal whether *P.m. hudsonianus* is the result of the reinvasion of the North American continent by *P. machaon* after the ice age or whether it is descended from a member of the *machaon* group that was present in America before the last glaciation. It is conceivable that it might even be due to an interglacial colonization.

The *machaon* group of Swallowtails offers very suitable material for an analysis of evolution in a group of closely related organisms and may well shed valuable light on the process of speciation in general.

Summary

1. The present investigation has confirmed the hypothesis that the difference between the orange spots on the larva of *P. machaon* and the yellow ones on the larva of *P.p. asterius* is controlled by a pair of allelomorphs, yellow being recessive or nearly recessive. However, when the subspecies *P.m. hippocrates* is one of the parents the spots are not orange as they are with other subspecies but are pale orange and thus intermediate between the two colours in appearance. The larva of the F_1 hybrid between *P.p. asterius* and *P. hospiton* had yellow spots and not orange or red ones as did the larvae of *P. hospiton, P. machaon* and their hybrid.

2. In hybrids between *P. machaon* from Europe and *P.m. hippocrates* there is a marked excess of males (the homoga-

metic sex), a situation which has not been found among any other hybrids of the *machaon* group so far investigated except possibly the hybrid between *P.m. hippocrates* and *P.p. asterius*.

3. The difference between the colour pattern of larvae of *P.m. saharae* and other subspecies of *P. machaon* appears to be controlled by a pair of allelomorphs with the heterozygotes intermediate in appearance. The abnormal colour pattern of the larvae of *P.m. saharae*, together with the fact that hybrids involving this subspecies tend to show abnormal development in the F_2 generation, lends support to the hypothesis that *P. hospiton* originally came from North Africa whereas the subspecies of *P. machaon* which now inhabits Corsica and Sardinia came from Europe.

4. The larva of the hybrid between *P. machaon* and *P. bairdi* has been obtained and apparently resembles the larva of *P. bairdi*.

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EVOLUTION OF SOME ENDOCARPAL FEATURES IN THE TRIBE TINOSPOREAE (MENISPERMACEAE)

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INTRODUCTION

The geological record of the angiosperms is conspicuously deficient in specific information regarding the nature and direction of morphological changes in time within the various taxa. Our present knowledge of such changes is based almost exclusively upon comparative data derived from the study of modern plants. There is no well documented sequence among the flowering plants comparable, for example, to the Cenozoic record of the Equidae among the vertebrates. That such evidence is lacking for the higher plants is due in large part to the fact that, while the relationships of the angiosperms have been established chiefly on the basis of their reproductive structures, their known fossil record consists for the most part of leaves (Chaney, 1949). The most notable exception to this situation is the large assemblage of fruits and seeds constituting the Eocene London Clay flora of England (Reid and Chandler, 1933). Some of the London Clay forms represent previous stages in the evolution of the reproductive structures borne by modern members of their respective groups.

An unique assemblage of fossil dicotyledonous fruits showing distinct affinities with the London Clay flora has recently been described from a single locality in the Eocene Clarno formation of north central Oregon (Scott, 1954 and mss.). The Clarno fossils are preserved as chalcedony locule casts surrounded by the carbonized or silicified remains of the fruit walls; consequently, the morphology of the fruits and seeds has been retained in detail. As is true for the London Clay fruits, certain of the Clarno fruits and seeds provide direct evidence regarding the course of evolutionary modification in their respective taxa.

The Clarno assemblage includes an unusually high proportion of forms whose affinities indicate that they represent woody vines or lianas. Conspicuous among these lianas are members of the Menispermaceae, a geologically ancient family in which the morphology of the fruits and seeds has been utilized extensively by taxonomists in establishing the relationships among the modern representatives (Miers, 1864–1891; Diels, 1910). The extent to which the fruits and seeds of the living menisperms have been collected and studied has made it possible to compare the structural features of the fossil forms on an unusually broad basis.

Four of the five Clarno genera in the Menispermaceae belong to the tribe Tinosporeae, which includes some 19 extant genera of woody vines found chiefly in subtropical and tropical regions in both the Old and New Worlds. The Clarno representatives of the tribe include species belonging to the modern genera *Tinospora* Miers and *Calycocarpum* Nutt. In addition, the genera *Chandlera* and *Odontocaryioidea* have been proposed (Scott, 1954) for two forms differing markedly from any known living genus in the tribe.

The fruits in the Tinosporeae are one-seeded drupes with sclerified endocarps, fleshy mesocarps, and thin exocarps. The hard endocarp or inner layer of the

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fruit, comparable to the pit of a peach or cherry fruit, is typically elongate and more or less boat-shaped with a prominent depression, the condylar cavity, on the ventral surface. The endocarp may be smooth or it may be ornamented with projections or spines which are variously arranged. In one pattern occurring in several genera, the spines are aligned in longitudinal rows. In some other genera of the Tinosporeae there are one to three projections at each end of the endocarp. The projections have been interpreted as extensions developing from the surface of the endocarp (Diels, 1910). However, structural features of the extinct genera *Chandlera* and *Odontocaryioidea* suggest an alternate possibility: that these two arrangements of projections were derived by reduction from ancestral forms whose thick walled endocarps had relatively smooth exteriors but were partially hollow. The derivation of each type will be taken up separately.

Derivation of Projections in Rows

Evidence regarding the possible origin of longitudinally aligned projections is furnished by the Clarno genus *Chandlera*. This genus is based upon structurally preserved specimens of its seed and endocarp. The thick walled endocarp was a large (50 mm. long), oblong-ellipsoidal structure. A well developed system consisting of six lacunae separated by partitions extended longitudinally within its dorsal wall (fig. 1A). The median dorsal partition and the marginal partitions at the sides of the endocarp were much more regular than the two lateral partitions between them on each side of the dorsal surface. At places these irregular lateral partitions were discontinuous so that adjacent lacunae were connected (fig. 1B). Casts of this system of lacunae, together with the casts of the septate ventral condylar cavity, form a striking structure in the fossil state. The rounded cross sections of the lacunae casts show

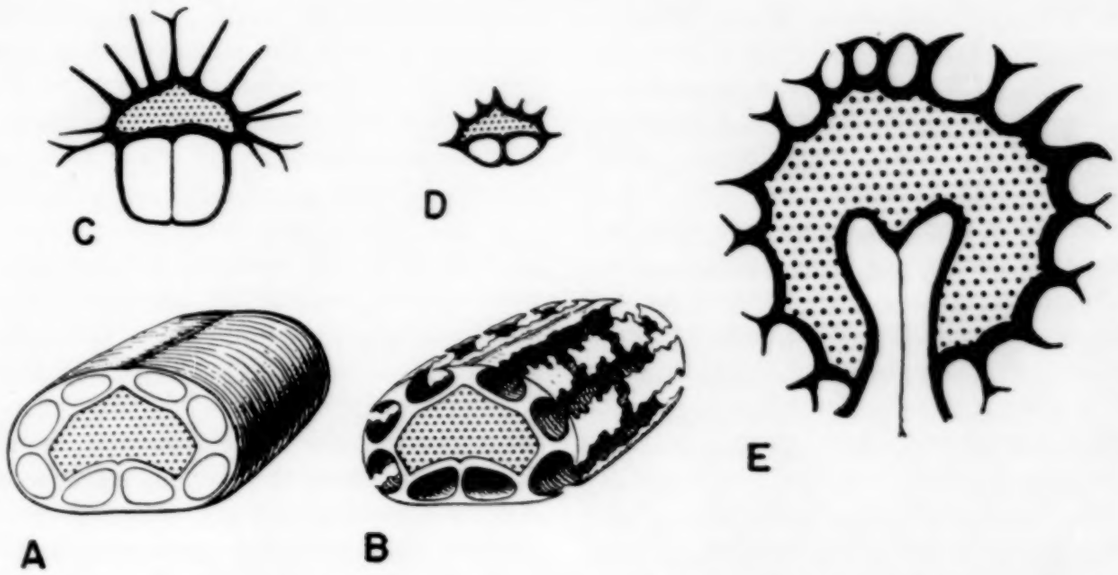

Fig. 1. Derivation of projections in rows. Locules are stippled in all diagrams. A. Cutaway view of the endocarp of *Chandlera lacunosa* exposing a transverse section. The two cavities beneath the locule represent the septate condylar cavity; the others are the lacunae in the endocarp wall. × 1. B. Hypothetical form derived from *Chandlera* by the reduction of the endocarp wall opposite the lacunae. × 1. C. *Parabaena megalocarpa*, transverse section through the endocarp. Note the bifurcated median dorsal and lateral spines. × 2. D. *Parabaena denudata*, transverse section through the endocarp. The rows of spines correspond in number and position to the interlacunal areas of *Chandlera*. × 2. E. *Chlaenandra ovata*, transverse section through the endocarp. Tips of the bifurcating spines in adjacent rows are fused in places. × 1.

that each partition between adjacent lacunae was thick at the base, narrowed outward, and then flared to form the continuous outer region of the endocarp wall.

The morphology of its seed and condyle show that the nearest affinities of *Chandlera* are with the modern Paleotropical genus *Parabaena* Miers, although neither *Parabaena* nor any other living genus of the Tinosporeae has endocarps with lacunae enclosed in their walls. However, several genera contain species with spinose endocraps, and in some of these the spines are aligned into rows. This is the case for *P. denudata* Diels and *P. sagittata* Miers. In both species, the rows of spines correspond in number and approximate position to the inter-lacunal partitions in the endocarp of *Chandlera* (fig. 1D).

Parabaena megalocarpa Merr., in several respects the species most like *Chandlera lacunosa,* has a lengthwise row of spines at the middle of the dorsal surface of its endocarp. Another row extends around the endocarp at its lateral margins. These rows correspond in position, respectively, to the median dorsal partition and the marginal partitions of *Chandlera.* Each of these rows might be described more properly as a wall or plate, for it consists of a continuous unit of sclerified tissue that terminates in projections along its outer margins. The projections are composed of aligned fibers that are continuous with those forming the plate from which they extend. In addition to the median dorsal and lateral rows, there are numerous irregular spines on the sides of the dorsal surface. A number of the spines in the row at the lateral margins of the endocarp of *P. megalocarpa* are branched dichotomously at their tips. These branches extend laterally at right angles to the row on which they are borne (fig. 1C).

An analogous situation occurs in the fruits of *Chlaenandra* Miq., a monotypic genus which, like *Parabaena megalocarpa,* grows in New Guinea. The large, subglobose endocarp of *Chlaenandra* bears several partially fused rows of projections whose tips show marked dichotomous branching. Not only are the tips branched, but some of the branches from adjacent rows of projections are joined. This lateral union forms a continuous outer layer that for short distances roofs the space between the rows (fig. 1E). Where it exists, this continuity between adjacent rows of projections creates a structure similar to that enclosing the lacunae of *Chandlera.*

The linear arrangement of the projections and their terminal dichotomy in *Parabaena* and perhaps other modern genera of the Tinosporeae readily can be visualized as having developed from a lacunose endocarp like that of *Chandlera.* If the thin regions of the outer wall above the lacunae did not become sclerified during the development of the fruit of *Chandlera,* the partitions would remain as apparently bifurcating plates arranged in rows (fig. 1B). Further reductions in the pattern of sclerification would result in a spinose appearance. This derivation explains at once the occurrence in some species of projections consisting in part of plates rather than spines, appearing to branch dichotomously at their tips, and arranged in longitudinal rows.

It has been noted that in some species of *Parabaena* the spines are arranged in rows corresponding in position to the interlacunar partitions of *Chandlera.* However, other species may have a less regular arrangement of spines. In *P. megalocarpa,* for example, the median dorsal and marginal spines are in rows, but the lateral spines are irregular. This pattern does not offer any serious difficulty to the present interpretation, for irregularity in the arrangement of the lateral spines is present, although to a lesser degree, in *Chandlera.* The lateral partitions of the fossil genus were variable in width and position. They also were discontinuous in contrast to the continuous dorsal and marginal partitions, (fig. 1B).

The morphology of the endocarp of the extinct *Chandlera* furnishes a basis for interpreting the tendencies toward alignment, bifurcation, and coalescence of the spines in some modern species of the Tinosporeae as relic features. The plates and spines represent remnants of the interlacunar partitions of thick endocarps whose walls contained either lacunae or areas of unmodified parenchyma tissue. If this interpretation is correct, the endocarpal ornamentation of at least some members of the Tinosporeae has originated by reduction from lacunose endocarps rather than by elaboration of originally smooth, thin-walled endocarps.

Although the lacunose endocarp of *Chandlera* appears to represent an ancestral structural type in the Menispermaceae, it is significant that among the London Clay forms not yet published is a member of the tribe Tinosporeae in which the endocarp bears rows of spines (letter from M. E. J. Chandler dated Sept. 25, 1953). Because the London Clay formation is older than the Clarno, it is apparent that the evolutionary processes that led to the production of spines in the tribe predate the Clarno occurrence of *Chandlera*. Consequently, this extinct genus is best regarded as a relic form with primitive features rather than as the direct antecedent of a particular modern genus.

Derivation of Basal and Apical Projections

The Clarno genus *Odontocaryioidea* provides a basis for suggesting the origin of the characteristic basal and apical projections found on the endocarps of several modern genera of the Tinosporeae. This extinct genus is related to the modern *Odontocarya* Miers, which is found in the West Indies and from Panama to the Amazon Basin. In addition, as described later in this paper, a definite relationship exists to unidentified material of the tribe from New Guinea. The relationship between the fossil form and *Odontocarya* is shown by the similarity in the structure of their seeds. The small (up to 14 mm. long), boat-shaped endocarp of *Odontocarya* has a conspicuous condylar cavity on the ventral side. It has one median and two lateral projections or "teeth" at each end (fig. 2F), this feature having suggested the generic name.

Molds of the larger (18–25 mm. long) endocarps of *Odontocaryioidea* show that they were regular in profile rather than dentate at the ends. These fossil endocarps differ from those of any living genus in the Tinosporeae by containing prominent cavities at both base and apex (fig. 2B). These cavities must have had openings externally, for during burial the matrix material in which the endocarps are imbeded filled the cavities to produce, in the fossil state, nodules at base and apex (fig. 2A). There are always two nodules at the apex of the endocarp, one on each side. At the base there also may be two nodules, but more frequently only one is present. Where there is but a single basal nodule, it is reniform with a broad, median depression on the ventral side. This depression establishes the presence, in the living state, of a tapering median ridge representing a remnant of the partition that once separated two distinct cavities.

It appears likely that the tridentate endocarp of *Odontocarya* was derived from an endocarp like that of the fossil genus by the elimination of the thin portion of the endocarp wall that enclosed the dorsal and ventral sides of the cavities at base and apex. The remaining median and marginal regions would constitute projections like those of the modern genus. A decrease in size and a change in the proportions of the endocarp have accompanied the reduction of the endocarp wall. This origin is shown diagrammatically in fig. 2C, D, F.

If the derivation suggested for modern tridentate endocarps is correct, the morphology of the Clarno form has a surprisingly general significance in the Tino-

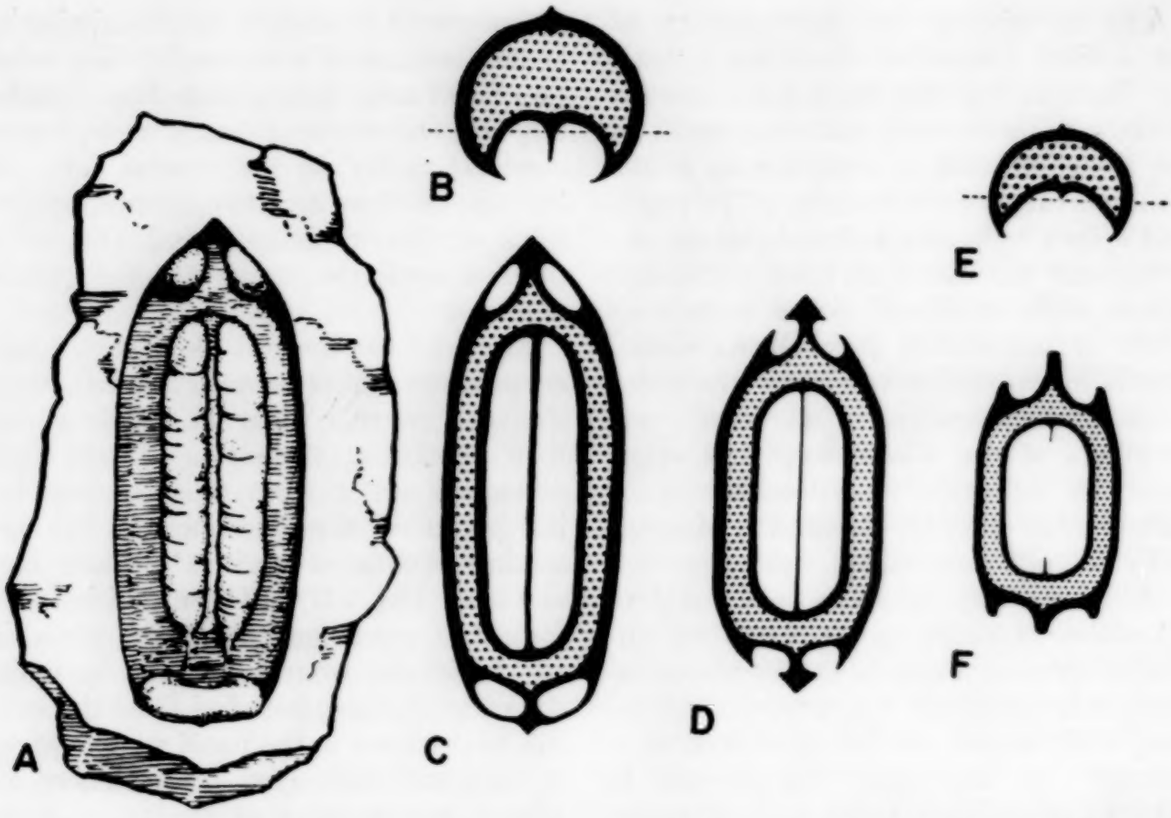

FIG. 2. Derivation of basal and apical projections. Locules are stippled in all diagrams. A. Cast of the ventral side of the endocarp of *Odontocaryioidea nodulosa* showing basal and apical nodules and the elongate cast of the open condylar cavity. × 2. B. *O. nodulosa,* transverse section through the endocarp. The dashed line through the condylar cavity shows the plane of section in C. × 2. C. *O. nodulosa,* longitudinal section through the endocarp showing basal and apical cavities. × 2. D. Hypothetical intermediate showing reduction of endocarp wall opposite the cavities and decrease in size. × 2. E. *Odontocarya nitida,* transverse section through the endocarp. The dashed line through the condylar cavity shows the plane of section in F. × 2. F. *O. nitida,* longitudinal section through the endocarp showing basal and apical projections. × 2.

sporeae. Genera with basically similar tridentate endocarps are found in each of the major tropical regions of the world. In each region, the genus regarded as the most primitive on comparative evidence (Diels, 1910) is the genus possessing endocarps most similar to those of the fossil genus.

Odontocarya is considered by Diels to be the most primitive member of the Tinosporeae in the Neotropics. It is one of three closely related American genera, the others being *Somphoxylon* Eichl. and *Synandropus* A. C. Smith. The fruit of *Synandropus* is not known and it has been described for only one species of *Somphoxylon.* The endocarp of this species is similar to that found in *Odontocarya,* although the dentate projections are less prominent. It differs chiefly in that the condylar cavity, open in *Odontocarya* and the extinct genus, is covered by extensions of the endocarp wall which meet along a median slit.

In addition to these American genera, representatives of the tribe Tinosporeae are found in tropical Africa and Madagascar, Southeast Asia, and Australia. The endocarps of the African *Desmonema mucronulatum* Engl. are not tridentate at the apex. However, a prominent median apical projection is always present, and the base of the endocarp is typically tridentate. The dorsal surface bears four small extensions, two near the base and two near the apex (Diels, 1910, fig. 52 J). These extensions are located in the proper position to represent remnants of the

portions of an endocarp wall enclosing cavities like those found in the extinct genus. Comparable extensions also occur in the African genus *Chasmanthera* Hochst. *Platytinospora* Diels also has the sagittate longitudinal profile characteristic for *Desmonema* although it lacks lateral projections at the apex. *Desmonema* is regarded by Diels as the most primitive among the African members of the tribe.

Endocarps with tridentate apices or bases are rare among the Asiatic genera of the Tinosporeae. *Tinospora*, recognized by Diels as the most primitive genus in this region, is also the most widespread. It consists of some 25 species distributed in Southeast Asia, Australia, and Africa. Fruits belonging to *Tinospora* typically have endocarps with prominent median projections at base and apex. The longitudinal axis of these endocarps is slightly curved rather than straight as it is in *Odontocaryioidea*. However, it is not difficult to hypothesize their derivation from a hollowed endocarp like that of the Clarno genus by continued reduction leading to elimination of the lateral projections at both ends of the endocarp.

The tridentate form which necessarily would have been an intermediate in such a progression is represented by *Tinospora polygonoides* Diels from New Guinea. This is the only species of the genus described by its author as having three projections at both base and apex of its endocarp. Material known to represent this species was not available for this study. However, among the unnamed material of the Menispermaceae in the Herbarium of the Arnold Arboretum is a single sheet, van Leeuwen 10708, bearing fruits collected in New Guinea. The endocarps in this collection are prominentaly tridentate at both ends and are less curved along the longitudinal axis than is usual among species of *Tinospora*. The exact taxonomic position of this plant cannot be determined with the material at hand. Whether this collection represents *T. polygonoides* or another species, it serves to establish the presence of a member of the Tinosporeae with conspicuously tridentate endocarps in Austro-Malaysia.

The preceding discussion has brought out the present day distribution of genera with tridentate endocarps in the three major tropical regions of the world. The structural similarity among these endocarps suggests the possibility that they evolved from a common ancestor. The morphology of the extinct *Odontocaryioidea* indicates that this genus represents the type of endocarp from which these modern plants could have been derived. Like *Chandlera*, *Odontocaryioidea* is best regarded as a relic form retaining ancestral features rather than as the direct precursor of any one modern genus.

Discussion

The occurrence of lacunae resulting from the distintegration of regions of parenchymatous tissue within the endocarp wall is limited in the Tinosporeae to extinct genera. Although no longer found in members of this tribe, such parenchymatous regions are not unique among the fruits of dicotyledonous plants. They occur in the bony inner layer of the fruit wall in genera of several families; e.g., *Juglans* (Juglandaceae), *Dracontomelon* (Anacardiaceae).

The presence of internal parenchymatous areas demonstrates that the term *endocarp*, as commonly applied to the inner, bony portion of the fruit wall, refers to a heterogeneous structure. As originally proposed (Richard, 1808), the term was applied only to the inner epidermis of the fruit. Use of the term in a broader sense tends to obscure the fact that the structures to which it refers often are not equivalent in different taxa. Variation exists in such features of the endocarp wall as the derivation of component tissues, orientation of cells in different layers, vascularization, and the amount of sclerification. For example, the pits of closely related species of *Prunus* differ markedly in structure (Sterling, 1953).

The variable nature of the so-called endocarp wall has been pointed out by Sterling (1953) who advocates a return to the original definition of Richard. The feasibility of eradicating the widespread use of the term in the broad sense is doubtful; however, it should be realized clearly that this usage merely designated an inner, predominately sclerified layer of the fruit wall without regard for its derivation and histologic composition.

The evidence from the fossil material at hand suggests that evolution of the fruits in the Tinosporeae has involved modification of the pattern of cellular differentiation within the endocarp. The modification consists of the failure of cells in certain regions to develop thick secondary walls and differentiate into sclerified tissue. That such changes in the pattern of endocarp differentiation actually have occurred can be demonstrated both in fossil and in living forms. It has been pointed out previously that *Odontocaryioidea nodulosa* may have either one or two basal nodules. The original difference between the double and single state consisted of the presence or absence of a central partition in the basal cavity. Evidently, the degree of sclerification of the cells in the region of the partition was variable. It has also been mentioned that the endocarps of the closely related genera *Odontocarya* and *Somphoxylon* differ chiefly in that the condylar cavity, open in *Odontocarya,* is enclosed by an extension of the bony endocarp wall in *Somphoxylon.*

The derivation of modern forms from endocarps like those of the Clarno genera appears to have been facilitated by the presence of lacunae in the fossils. Due to these lacunae, a quantitatively small alteration in the pattern of sclerification has markedly altered the appearance of the fruits. The originally thick walled endocarp has been modified into a thin walled, apparently ornamented structure. There is convincing evidence, based on diverse occurrences of unrelated groups (London Clay flora: Reid and Chandler, 1933; Brandon lignite: Barghoorn, personal communication, Feb. 10, 1955; Clarno fruits: Scott, 1954), that one of the major modifications of fruit structure in time has been a trend toward a reduction in the relative thickness of the walls of endocarps.

The suggested origin of endocarpal projections in some cases by reduction of thick walled endocarps does not preclude the possibility that equivalent structures have arisen by elaboration in other cases. There is no apparent reason why changes in the structure of the endocarp wall could not involve an increase rather than a decrease in the amount of sclerification. Future developmental and histological studies of a variety of drupaceous fruits may show that both processes are effective. However, the possibility of origin of endocarpal ornamentation by reduction should be taken into account in evaluating the relationships of groups with strongly ridged endocarps.

In any case, reduction of the endocarp wall seems to have played a significant part in the development of the structural features that characterize the fruits of some living members of the Tinosporeae. The suggested relationships of the genera *Chandlera* and *Odontocaryioidea* illustrate one of the uncommon cases among the angiosperms in which there is paleontological evidence regarding the evolution of morphological features.

It is significant from a phytogeographical standpoint that these two extinct genera, found in the New World, represent forms apparently ancestral to modern genera native to the Old World. This pattern reflects the fact that the Menispermaceae is an ancient family in which at least some of the genera were once more widely distributed. It is doubtful whether present distribution patterns are valid limiting factors in considering possible relationships in the Menispermaceae.

Summary

Drupaceous fruits belonging to two extinct genera in the tribe Tinosporeae

(Menispermaceae) occur in the Eocene Clarno formation of Oregon. The elongate endocarps of both genera contain lacunae; these are at the base and apex in *Odontocaryioidea* and in longitudinal rows in *Chandlera*. No extant genus in the tribe has lacunose endocarps. However, endocarps of the modern genera most closely related to *Odontocaryioidea* bear spines or projections at base and apex, and longitudinal rows of projections are found in modern forms related to *Chandlera*. These projections in the living forms have been regarded as ornamentation.

In each of the extinct genera, reduction resulting in elimination of the thin, outer region of the endocarp wall opposite the lacunae would produce structural features directly comparable to the projections found in modern forms. The paleontological evidence suggests the possibility that endocarpal ornamentation in at least some modern members of the Tinosporeae has originated from forms comparable to the Clarno genera by the reduction of thick-walled, lacunose endocarps rather than by the elaboration of thin-walled endocarps.

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GENETICS OF NATURAL POPULATIONS. XXV. GENETIC CHANGES IN POPULATIONS OF *DROSOPHILA PSEUDOOBSCURA* AND *DROSOPHILA PERSIMILIS* IN SOME LOCALITIES IN CALIFORNIA[1]

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INTRODUCTION

Evidence is accumulating that natural populations, not only of lower but also of higher organisms, undergo genetic changes which are sometimes surprisingly rapid. In insects which produce two or more generations per year the populations may be genetically different at different seasons; changes of a more enduring character are also known (reviews by Dobzhansky, 1951, and Andrewartha and Birch, 1954). But while the occurrence of such directly observable evolutionary changes is no longer unexpected, the causes which bring them about remain baffling even in the relatively better-studied cases (the moth *Panaxia,* Sheppard, 1953, the beetle *Harmonia axyridis,* Komai, 1954, red fox, Butler, 1951, species of *Drosophila,* Dobzhansky, 1952, to cite only few instances). In this field we are still in the data-gathering stage. Yet, it is needless to labor the point that the study of even slight microevolutionary changes taking place under natural conditions in wild species is fraught with interesting possibilities for an evolutionist.

Cyclic seasonal as well as year-to-year changes have been observed in *Drosophila pseudoobscura* on Mount San Jacinto in Southern California, and in this species as well as in *D. persimilis* in the Yosemite region of the Sierra Nevada of California (Dobzhansky, 1947a, 1948, 1952). Further observations were carried out in the latter region during the summer of 1954. It must be admitted that these observations raised more problems than they solved. Nevertheless, it seems desirable to report the results obtained to date, since there is no assurance that this work can be continued by the present writer.

MATERIAL

Collections of Drosophila have been made at Mather and at Aspen Ranger Station, Tuolumne County, California, in exactly the same neighborhoods as in previous years (Dobzhansky, 1948, 1952). The collecting techniques differed, inasmuch as larger trap cans and a variety of species of yeasts in the banana baits were used (Cooper and Dobzhansky, in press). These baits do not differ significantly in relative attractiveness for *D. pseudoobscura* and *D. persimilis,* or for the different chromosomal types of these species (Dobzhansky et al., in press). Wild females collected were allowed to produce progenies in individual cultures, and the salivary gland chromosomes were examined in a single larva from each progeny. The wild males were not used in this study because of the greater amount of labor necessary to determine the karyotype of a male.

SEASONAL CHANGES IN *D. pseudoobscura*

The percentage frequencies of the different gene arrangements in the third chromosomes of *D. pseudoobscura* found in the 1954 collections are shown in table 1 and figure 1. For comparable data for other years, see Dobzhansky, 1948 and 1952. Seven different gene arrangements occur in the Mather and Aspen Valley populations. Two of these, ST and AR,

[1] The work reported here has been carried out under Contract No. AT—(30-1)—1151, U. S. Atomic Energy Commission.

are very common, three arrangements, CH, TL, and PP, are moderately frequent, and two, SC and OL, are rare. Examination of the data shows that in 1954, as in previous years, the frequencies of ST chromosomes increased as the summer progressed, while the frequencies of AR gradually diminished. Other gene arrangements show no discernible trends of change. Statistical tests confirm this. The chi-squares for hetreogeneity for the different gene arrangements in the eight samples taken at Mather in 1954 are as follows:

Chromosome	Chi-Square	Probability
ST	27.44	<0.001
AR	39.90	<0.001
CH	7.18	0.40
TL	2.51	0.95
PP	4.32	0.75

The two samples taken at the Aspen Ranger Station (table 1) show an ostensible, but statistically not significant, increase of ST and decrease of AR from June to August. This agrees with what has been observed at Mather, and also at Aspen in previous years (Dobzhansky, 1948).

Comparing the Sierra Nevada and San Jacinto Populations

The chi-square tests show that the frequencies of ST and AR chromosomes are different in the different samples; they do not tell us whether the changes are irregular or exhibit definite trends. In accordance with the advice of Professor Howard Levene, regression lines have been calculated on the assumption that the rate of change is uniform during the period of observations. The regression equations turn out to be:

$$\text{Percentage of ST} = 20.66 + 0.1215\ X$$

$$\text{Percentage of AR} = 44.90 - 0.1540\ X,$$

where X is the number of days elapsed since June 2nd, which is the middle of the first period of observation (see table 1). The observed and the expected frequencies of ST and AR chromosomes are compared in table 2. The agreement is very good, as attested by the chi-squares (each of which has 6 degrees of freedom).

It appears, then, that the frequencies of ST chromosomes increase, and those of AR decrease, in the Mather population at approximately uniform rates from June to September. The observations made during the summers of 1946, 1947, 1950, and 1951 are, as far as they go, consistent with this conclusion. We must infer that during the winter season the frequencies of ST drop and those of AR increase, to make the changes cyclic. No observations are, unfortunately, available.

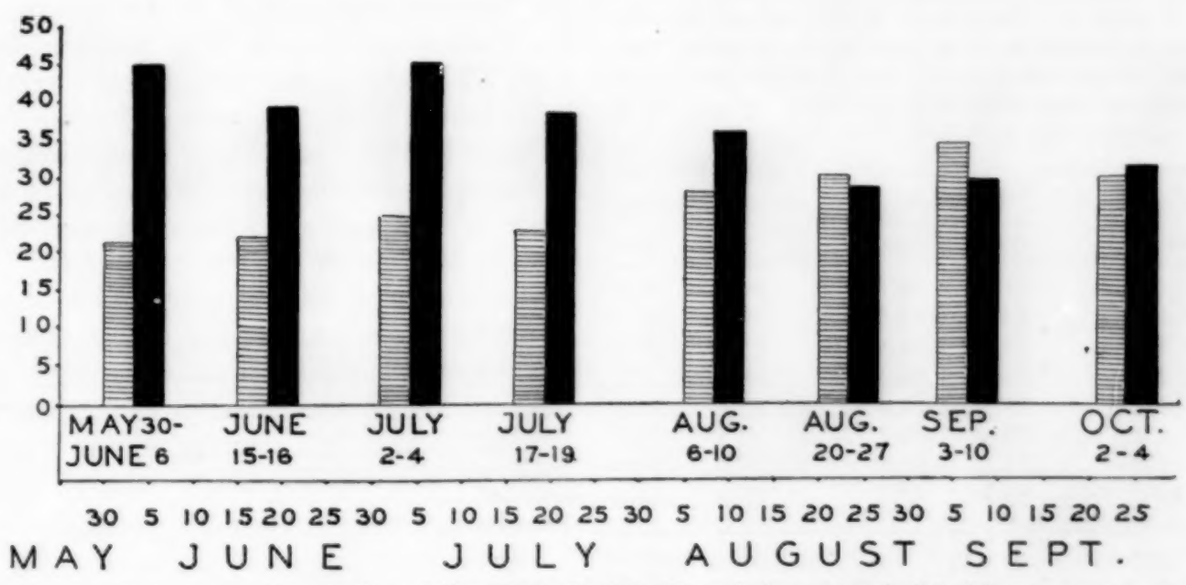

FIG. 1. Percentage frequencies of Standard (hatched) and Arrowhead (black) gene arrangements in the population of *Drosophila pseudoobscura* at Mather during the summer of 1954.

TABLE 1. *Frequencies (in per cent) of the gene arrangements in* Drosophila pseudoobscura *at Mather and at Aspen Valley*

ST—Standard, AR—Arrowhead, CH—Chiricahua, TL—Tree Line, PP—Pike's Peak, SC—Santa Cruz, OL—Olympic, n—number of chromosomes studied. Collections made in 1954.

Date	ST	AR	CH	TL	PP	SC	OL	n
Mather								
May 30–June 6	21.4	44.9	10.7	11.7	9.8	1.1	0.4	532
June 15–17	22.1	39.7	17.7	9.6	10.3	0.7	—	136
July 2–4	25.0	45.4	9.7	9.7	9.7	0.5	—	196
July 17–19	23.0	38.9	12.8	12.5	11.5	1.0	0.3	296
Aug. 6–10	28.3	35.3	11.6	10.7	11.6	—	—	346
Aug. 20–27	30.8	29.0	13.0	11.5	13.6	0.6	1.5	338
Sept. 3–10	34.4	29.8	12.7	9.9	10.6	1.4	1.2	416
Oct. 1–3	30.4	32.6	8.7	13.0	13.0	—	2.2	46
Aspen								
June 15–19	13.0	52.0	8.0	11.0	12.0	4.0	—	100
Aug. 23–28	23.1	42.3	13.5	9.6	9.6	1.9	—	52

The situation in the Mather population is clearly different from that in the populations on Mount San Jacinto, some 300 miles to the south. In the latter region, seasonal changes have been observed in the locality of Piñon Flat, but there the ST and CH, instead of ST and AR, chromosomes are involved. Moreover, the frequencies of ST decrease, and those of CH increase, from March to June, and a reversal occurs between June or July and September (Dobzhansky, 1947). Epling, Mitchell and Mattoni (1953) believed that the reversal occurred abruptly, "in what may have been a single generation," in at least some years. So rapid a change would be hard to explain by natural selection, but the difficulty is spurious because the sampling errors are large enough to conceal a quite gradual character of the changes. At the Keen Camp locality, some 15 miles away from Piñon Flat, the frequencies of ST, AR, and CH chromosomes show no detectable seasonal changes (Dobzhansky, 1947; Epling et al., 1953). Such differences in the genetic composition of local populations and in their reactions to the environments show a remarkable versatility of adaptive evolution.

TABLE 2. *Frequencies of ST and AR chromosomes observed at Mather during the summer, 1954, and frequencies expected on the assumption of a uniform linear regression with time.* June 2nd is considered the zero day.

	Standard		Arrowhead	
Days	Obs.	Exp.	Obs.	Exp.
0	21.4	20.7	44.9	44.9
14	22.1	22.3	39.7	42.7
33	25.0	24.1	45.4	39.8
46	23.0	26.3	38.9	37.8
67	28.3	28.8	35.3	34.6
83	30.8	30.7	29.0	32.1
97	34.4	32.4	29.8	29.9
120	30.4	35.2	32.6	26.4
Chi-Square = 3.11		Chi-Square = 5.55		
Probability = 0.8		Probability = 0.5		

SEASONAL CHANGES IN *D. persimilis*

The commonest gene arrangement in the third chromosome of *D. persimilis* in the Yosemite region is WT, followed by KL; ST and MD are rare but are regularly found, while SE and CO occur in isolated individuals. The composition of the samples collected in 1954 is shown in table 3. Inspection of the data suggests that at Mather WT chromosomes fell in frequencies in June and July, and rose in August and September; KL chromosomes underwent changes opposite in sign to those of WT. The chi-squares for heterogeneity in the eight samples lited in Table 3 are as follows:

Chromosome	Chi-Square	Probability
WT	26.97	<0.001
KL	18.16	0.01

TABLE 3. *Frequencies (in per cent) of the gene arrangements in* Drosophila persimilis *at Mather and at Aspen Valley*

WH—Whitney, KL—Klamath, ST—Standard, MD—Mendocino, SE—Sequoia, CO—Cowichan, n—number of chromosomes studied. Collections made in 1954.

Date	WH	KL	ST	MD	SE	CO	n
Mather							
May 30–June 6	80.9	12.1	3.9	3.1	—	—	388
June 15–17	75.0	17.4	2.8	4.7	—	—	212
July 2–4	75.0	16.7	2.4	5.9	—	—	84
July 17–19	73.0	18.0	4.0	5.0	—	—	100
Aug. 6–10	73.1	19.2	3.2	3.8	0.6	—	156
Aug. 20–27	79.1	15.3	1.4	4.2	—	—	426
Sept. 3–10	84.2	11.2	2.1	2.4	—	0.1	1098
Oct. 1–3	85.0	11.3	3.1	0.6	—	—	160
Aspen							
June 15–19	84.9	10.1	2.4	2.4	0.3	—	149
Aug. 23–28	84.9	7.5	2.7	4.8	—	—	146

The character of the seasonal changes is, however, quite different in *D. persimilis* from what it is in *D. pseudoobscura*. In the latter species the changes proceed from June to September inclusive at apparently uniform rates (see above). In *D. persimilis*, the frequencies of WT chromosomes fall during June and July and increase during August and September. KL chromosomes wax in frequency in early summer, and wane in late summer (Figure 2). In the absence of evidence to the contrary, we need not suppose that any changes occur during the cold season.

Curiously enough, the seasonal changes

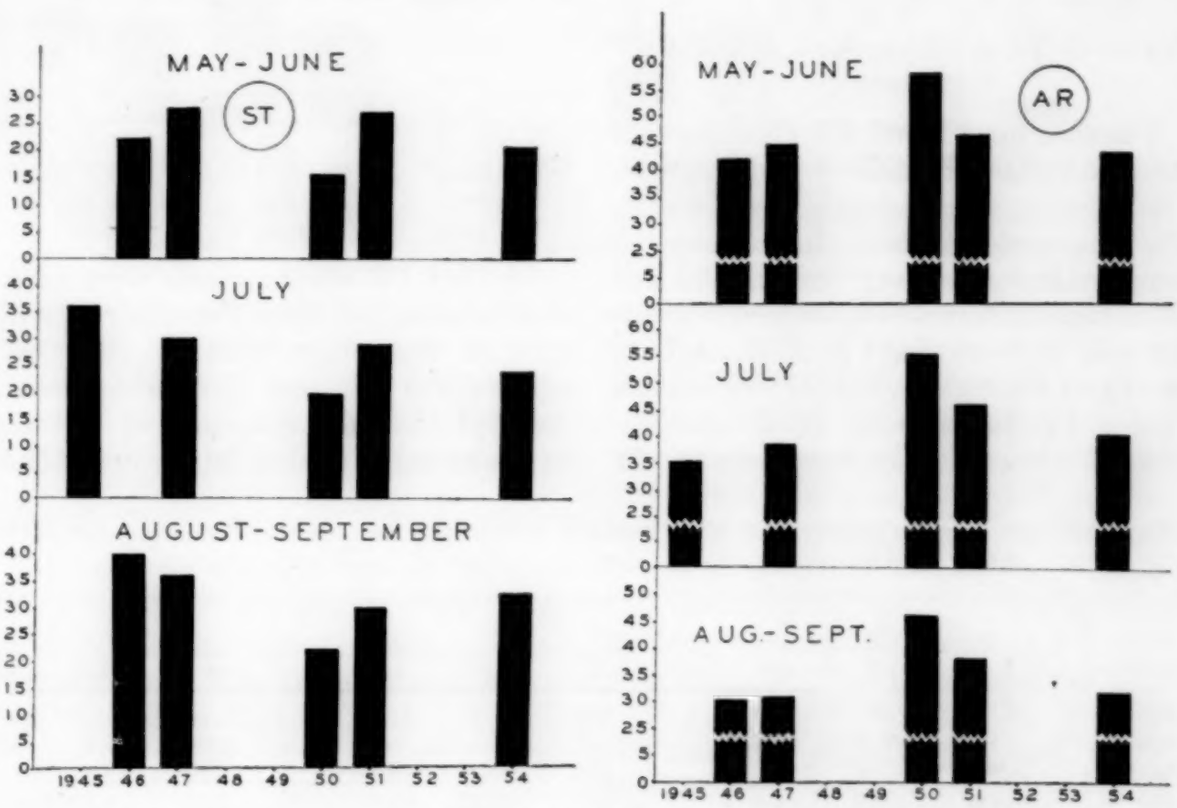

FIG. 2. Percentage frequencies of Standard (left) and Arrowhead (right) chromosomes in the population of *Drosophila pseudoobscura* at Mather in different months and different years of observation (1945–1954).

observed in the Mather populations of *D. persimilis* in 1954 are not paralleled in the more limited data available for this species and locality in 1946, 1947, and 1950 (Dobzhansky, 1948 and unpublished). In 1951, when the species was much less abundant at Mather than it was in 1954, the following percentages of the chromosomes of different kinds were observed:

Date	WT	KL	ST	Others	n
June 25–July 8	64.3	17.1	15.7	2.9	70
Aug. 7–16	69.6	21.7	6.5	2.2	46
Aug. 23–26	82.7	6.4	9.0	1.9	156

It is possible that seasonal changes in the population of Mather occur in some years but not in others; another possibility is that the population changed genetically during less than a decade it was under observation. There is no way at present to discriminate between these possibilities as far as the seasonal changes are concerned. It will, however, be shown below that the populations do change from year to year.

Year-to-Year Changes in *D. pseudoobscura*

The data for ST and AR chromosomes are summarized in table 4 and figure 2. The situation is particularly clear for AR. The frequencies of these chromosomes at comparable seasons were low in 1945 and 1946, increased in 1947, reached a maximum in 1950, declined in 1951, and returned to almost the 1945–1946 level in 1954. The behavior of ST is less consistent. However, the frequency of ST was the lowest in 1950 in every season; this is the year when AR was commonest. The highest values of ST were observed in 1947 (May–June), 1945 (July), and 1946 (August–September). The 1954 values of ST are higher than those for 1950, but below those for 1945–1946 (except in May–June).

On the whole, the data are consistent with the supposition that from 1945 to 1950 there was a trend in the Mather population towards reduction of the frequencies of ST and increase of AR; the trend became reversed between 1950 and 1954. Because of the several missing years there is no way to tell whether these trends were or were not regular (fig. 2). The data for localities in the Yosemite region other than Mather are too scanty to be relied upon, but there are good indications that the Aspen population underwent changes parallel to those at Mather.

A quite independent, and in a way more spectacular, trend in the Mather population concerns the PP gene arrangement. The data are as follows:

Year	Chromosomes studied	Per cent PP
1945	308	0.00
1946	336	0.30
1947	806	0.62
1950	812	2.83
1951	856	4.55
1954	2306	11.06

In 1945 PP chromosomes were ostensibly absent, but since then they steadily rose in frequency, until in 1954 they equaled the TL and CH chromosomes. In 1945–1947 the frequency of CH was in the neighborhood of 20 per cent (Dob-

TABLE 4. *Frequencies (in per cent) of ST and AR gene arrangements in the Mather population of* D. pseudoobscura *in different years; n—number of chromosomes studied*

	May–June			July			August–September		
Year	n	ST	AR	n	ST	AR	n	ST	AR
1945	—	—	—	380	35.7	35.7	—	—	—
1946	178	21.9	42.7	—	—	—	158	39.9	30.4
1947	262	27.5	45.0	304	30.3	38.2	240	35.8	31.2
1950	180	16.1	57.8	106	19.8	55.7	526	21.9	45.8
1951	100	27.0	47.0	416	29.3	46.2	340	29.7	38.5
1954	668	21.6	43.9	492	23.8	41.5	684	29.5	32.2

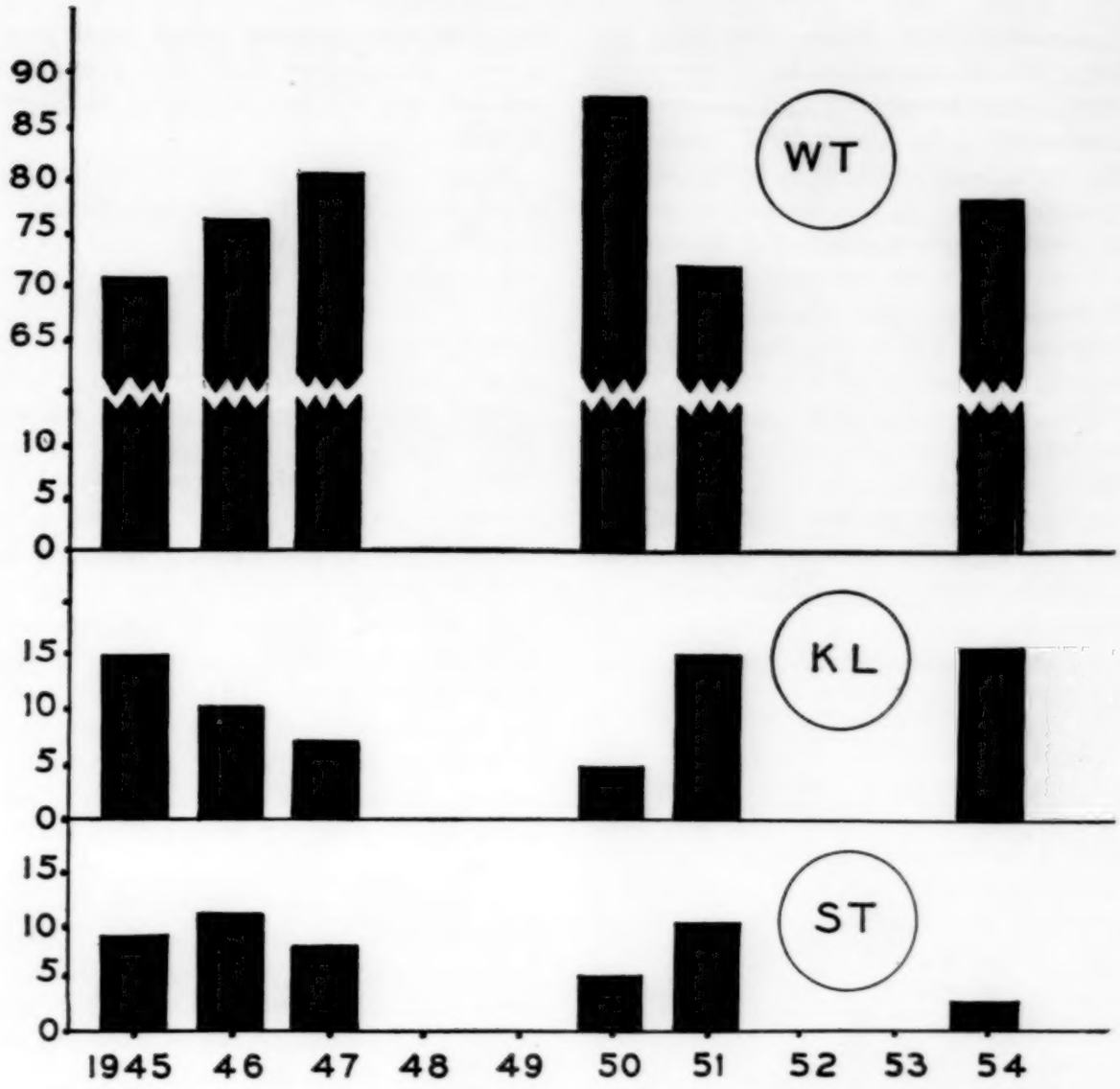

FIG. 3. Percentage frequencies of WT, KL, and ST chromosomes in the population of *Drosophila persimilis* at Mather in different years of observation (1945–1954).

zhansky, 1948, table 3), so that as PP increased in frequency CH lost ground. There might have been also a slight increase in the frequency of TL between 1947 and 1954, but this is not certain.

YEAR-TO-YEAR CHANGES IN *D. persimilis*

Summing up the data for the Mather population of *D. persimilis* in 1954 (table 3) we obtain the following average frequencies of the gene arrangements in the third chromosomes:

WT	KL	ST	MD
80.6	13.4	2.7	3.2

The status of this population in 1954 may now be compared with that in previous years. Such a comparison is shown in figure 3 (for numerical data for the years 1945–1951, see table 3 in Dobzhansky, 1952). Quite significant changes have taken place during the period of observation. The frequency of WT, which is the commonest chromosome structure, rose from 1945 to 1950, fell in 1951, and rose again slightly in 1954. This behavior is, then, reminiscent of that of AR chromosomes in *D. pseudoobscura* (see above), except for the rise in 1954. In contrast, KL chromosomes fell in frequency from about 15 per cent in 1945 to only 5 per cent in 1950, and rose again to 13 per cent in 1954. The behavior of KL is, then, reminiscent of that of ST in

D. pseudoobscura during the same period. ST chromosomes in *D. persimilis* varied more irregularly but nevertheless significantly. In 1946, 1947, and 1950 they were about as common in the Mather population as KL, but instead of becoming more frequent when the trends for WT and KL were reversed (see above), ST reached an all-time low in 1954. Other chromosomes (MD, SE, and CO) were too rare to yield significant data.

The Aspen population (table 3 of the present article and table 4 in Dobzhansky, 1952) underwent changes apparently parallel to those in the Mather population, although the data are not extensive enough to stand up by themselves.

Correlation with Rainfall

In the Sierra Nevada, as in much of California, summers are usually arid, while precipitation is relatively abundant from approximately November to April. Most of the breeding season of *D. pseudoobscura* coincides with the arid part of the year. The food sources of the fly depend on the state of vegetation, which is in turn influenced by the moisture accumulated in the soil. Now, the winter seasons (1946–47, 1947–48, 1948–49, and 1949–50) had less than average rainfall and snowfall while the seasons 1944–45, 1945–46, and 1950–51 had higher than average precipitation (figure 2 and table 5 in Dobzhansky, 1952). The drought years showed rising frequencies of AR chromosomes in *D. pseudoobscura* and of WT chromosomes of *D. persimilis*, while the more humid years brought higher frequencies of ST in *D. pseudoobscura* and of KL in *D. persimilis*. A "possible correlation with the climatic changes" was, accordingly, suggested by Dobzhansky (1952). Epling et al. (1953) rejected this hypothesis, because they found an only imperfect parallelism between the amount of precipitation and the changes in the chromosome frequencies in *D. pseudoobscura* at Keen Camp, in southern California. In that population the gene arrangements which show year-to-year changes in frequencies are ST and CH, not ST and AR as in the Sierra Nevada.

Ranger Evans, of the Yosemite National Park, has very obligingly placed at the disposal of the writer the data on the precipitation at the three weather stations close to Mather, namely Hetch-Hetchy, Lake Eleanor, and Yosemite Valley. The season 1951–52 had much greater than average precipitation, while the seasons 1952–53 and 1953–54 were close to the averages. Now, the populations of *D. pseudoobscura* and *D. persimilis* at Mather have by 1954 nearly restored the 1945–47 composition with respect to the frequencies of AR and ST chromosomes in the former and WT and KL chromosomes in the latter. The tentative working hypothesis that these changes stand in a causal relationship with the succession of drought and humid years is certainly worth keeping in mind for further testing.

It should be self-evident that this hypothesis does not imply that the changes in the relative frequencies of the different kinds of chromosomes in Drosophila populations are caused directly by the amount of moisture which falls on the flies. Similarly, the temperautre is not the only factor which brings about the seasonal cycles in Drosophila populations (see, however, Epling et al., 1953). Precipitation and temperature modify the biotic environments of the flies in countless ways, and the biotic modifications may well determine the adaptive values of the karyotypes of which the Drosophila populations are composed. Thus, the excellent work of Birch (1955) has suggested a possible solution of the long-standing puzzle of the seasonal cycle of the chromosome frequencies in *D. pseudoobscura* at Piñon Flat, in southern California. In this population CH chromosomes increase, and ST chromosomes decrease, in frequencies from March to June, and the change is reversed later in the summer. The summer part of the cycle has been

easily reproduced in experimental populations (Dobzhansky, 1947b), but it remained for Birch to show that CH/CH homozygotes may be fitter than ST/ST homozygotes when larvae are uncrowded and the population is expanding, as it is during the spring months at Piñon Flat.

Observations at Mather, in the Sierra Nevada, have shown that the relative abundance of species of Drosophila varies with season and from year to year. This is evidence of biotic changes in the fly environments. Thus, the abundance of *D. azteca* generally increases relative to that of *D. pseudoobscura* and *D. persimilis* from early to late summer (table 6 in Dobzhansky, 1952; table 3 in Cooper and Dobzhansky, in press). The abundance of *D. pseudoobscura* relative to *D. persimilis* varies within wide limits (table 7 in Dobzhansky, 1952; table 4 in Cooper and Dobzhansky, in press). *D. persimilis* generally builds relatively large populations early and late in the season, while *D. pseudoobscura* is most successful in July and August. In 1951 *D. persimilis* was, however, excessively rare—about 10 per cent of the combined catch of *D. pseudoobscura* and *D. persimilis*. Conversely, in 1954 *D. persimilis* was more abundant than *D. pseudoobscura* in June and in September. Facts such as these may certainly be relevant for understanding of the changes observed in the genetic composition of the Drosophila populations, although at present we are unable to see any obvious correlations.

Behavior of Chromosomes with PP Gene Arrangement

The complexity of the selective forces acting on a natural population is usually so great that any single environmental factor is not likely to be responsible for all the genetic changes that may occur. Apparently uncorrelated genetic changes are taking place in the same population at the same time. As shown above, in the Sierra populations of *D. pseudoobscura* there was a trend towards higher frequencies of AR and lower frequencies of ST chromosomes between 1945 and 1950. This trend became reversed between 1950 and 1954. But in the same populations there was an apparently steady progression of the frequencies of PP chromosomes during the entire period of observation (1945–1954).

No PP was encountered among 308 third chromosomes from Mather examined in 1945, one PP among 336 chromosomes in 1946, several in 1947, while in 1954 the frequency of PP rose to about 11 per cent of the total (see above). Prior to 1945, PP has been found as a rather rare gene arrangement in some populations of central California; the region where it is common or even dominant lies east and southeast of California, particularly in the Rocky Mountains, Texas, and northern Mexico (Dobzhansky, 1944).

To appreciate the extraordinary behavior of PP it is necessary to keep in mind that PP chromosomes have been increasing not only in the Yosemite region of the Sierra but also on San Jacinto, in southern California. No PP have been detected among the approximately 14,000 chromosomes examined by the writer from San Jacinto between 1939 and 1946 (Dobzhansky, 1947a), nor in the samples taken by Epling et al. (1953) in 1948, 1949, and 1950. Epling et al. encountered several PP chromosomes in samples from San Jacinto in 1951, and in 1952 and 1953 such chromosomes became fairly common, apparently at the expense chiefly of CH chromosomes, just as they did in the Sierra Nevada. Unfortunately, it is not known whether PP chromosomes appeared in other localities in southern California at about the same time as they did in the Sierra and on San Jacinto. However that may have been, a sudden and rapid increase of the frequencies of PP has taken place in places as different and as far apart as the Yosemite region and San Jacinto. This is perhaps the most spectacular change ever recorded in the genetic com-

position of natural populations of a wild species, apparently not under the influence of man-made changes. The spread of "industrial melanism" in some species of Lepidoptera represents, of course, an even greater change, but it is man-caused.

Rarity of the Mutational Origin of Chromosomal Inversions and Causes of Their Spread in Natural Populations

While the changes in the incidence of ST and AR chromosomes in the Sierra populations of *D. pseudoobscura* may have been due to climatic fluctuations, the causation of the spectacular rise of PP chromosomes is quite obscure. It is nevertheless useful to consider briefly two false leads which may suggest themselves to the unwary. These are (a) that PP chromosomes may have arisen by mass mutation, and (b) that they may have been introduced in the California populations from elsewhere.

This writer estimates that he has examined at least 47,000 chromosome sets in the salivary gland cells of larvae in the offspring of *D. pseudoobscura* and *D. persimilis* collected in nature. The third chromosomes have been examined more carefully than the rest, but minor changes in the gene arrangement might have been overlooked even in the third. Some gene arrangements were encountered only once in this material, and they may have represented recently arisen mutants. More precisely, each of the five inversions were seen in only a single individual (3 in the third, 1 in the second, and 1 in the fourth chromosomes). It does not follow, however, that these 5 inversions had arisen by mutation in the immediate ancestry of the individuals in which they were found. Most likely, at least some of them were rare inversions which had existed for many generations in the natural populations before being picked up. Indeed, some other inversions which were discovered originally in single chromosomes were found again when fresh samples were examined from the same populations.

More critical data for an estimation of the spontaneous mutation rates producing inversions came from the examination of the chromosomes in experimental populations kept in the laboratory. The chromosomes in the foundation stock of such experimental populations are examined within usually a small number of generations before the start of the experiments. Any variations in the gene arrangements observed in experimental populations have probably arisen in these populations themselves, or at least in their not-too-remote ancestors. To date, this writer has examined certainly more than 100,000 (and possibly twice that number) of chromosome sets in larvae from experimental populations (counting, of course, one set per larva). In one population several larvae showed a small terminal or subterminal deficiency in the third chromosome. In another population, a single larva had a short inversion in the fourth chromosome.

Spontaneous origin of new inversions in Dorsophila in nature is evidently rare. Although some strains of Drosophila may contain genetic variants which increase the breakability of the chromosomes, mutation rates which would have to be assumed to account for the spread of PP chromosomes unassisted by natural selection in California populations would be altogether fantastic. The same objection applies with almost equal force to the supposition that PP chromosomes were brought to California from the East, for example from Texas, by storms or similar events. That stray individuals may from time to time be so introduced in a population from far away is conceivable, although not proven. But this would not even begin to explain the sustained rise of the incidence of PP chromosomes in several localities in the Yosemite and the San Jacinto regions of California over a period of several years.

The spread of PP chromosomes has evidently been impelled by natural selec-

tion. But if so, there is no need to assume that PP arose by mutataion, or was introduced from the outside, in California localities between 1940 and 1950. PP chromosomes had almost certainly been present in populations of these localities with subliminal frequencies. These chromosomes carry a gene complex which, in certain environments, confers a high adaptive value on heterozygotes of PP with other chromosomes present in the Sierra and in San Jacinto populations. Just what changes in the environment favored the PP heterozygotes from about 1945 on is for the time being a tantalizing puzzle. It is the more puzzling since the same or similar changes appear to have taken place within a few years, and maybe simultaneously, both in the Yosemite and in the San Jacinto regions.

The observed changes in the incidence of PP may be an instance of a kind of evolutionary change which is both common and important. A chromosome with a given gene arrangement may contain, or may evolve by mutation and recombination, a linked gene complex which is useful in a certain range of environments. When these environments become available, owing to a climatic change or to other causes, the chromosomal type finds its opportunity and increases in frequency. The coadaptation of the gene complex in this chromosomal type with gene complexes in other types of chromosomes in the same population, is gradually perfected by natural selection. The components of the chromosomal pool of a population thus become altered and a reconstruction of the whole population genotype gradually ensues. The existence in a population of a variety of gene complexes in the chromosomes having the same gene arrangement, has been demonstrated by Cordeiro and Dobzhansky (1954) for *D. willistoni,* and by Dobzhansky, Pavlovsky, Spassky, and Spassky (1955) for *D. pseudoobscura.* The function of chromosomal inversions is simply to guard the integrity of the gene complex from too frequent destruction by recombination.

Summary

Populations of *Drosophila pseudoobscura* in the Yosemite region of the Sierra Nevada of California show seasonal changes in the relative frequencies of ST and AR chromosomes. During the summer of 1954 significant changes in the frequencies of certain chromosomal types have been observed also in the population of *D. persimilis* in the same region, although such seasonal changes were not observed in this species in previous years.

During the period of observation (1945–1954 with 4 years missing), the frequencies of AR chromosomes first increased and later decreased from year to year in *D. pseudoobscura,* while ST chromosomes showed the opposite change. In *D. persimilis* during the same period WT chromosomes first increased and then decreased in frequency, KL chromosomes showing the opposite change. These changes may have been produced by climatic fluctuations, namely by a succession of dry and wet years. This tentative explanation is not regarded as proven.

During the same period of frequency of chromosomes with PP gene arrangement rose in *D. pseudoobscura* populations of the Yosemite region from an ostensible zero per cent in 1945 to 2.8 per cent in 1950 and to 11 per cent in 1954. The gradual rise of PP was at the expense chiefly of CH gene arrangement. The causes which brought about this spectacular increase of the incidence of a genetic variant in the population both in the Yosemite region, and also on Mount San Jacinto, farther to the south, are unknown. Arguments are presented which show that the phenomenon cannot be explained by mutational origin or by introduction of PP chromosomes into California from elsewhere.

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SEMILETHAL COMPLEMENTARY FACTORS IN A TOMATO SPECIES HYBRID[1]

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INTRODUCTION

Complementary factors are encountered so frequently in genetic studies that they need not be considered as novelties by themselves unless they have some bearing on other problems. Since the discovery by Bateson (1907) of such interactions in peas, the work of Atwood and Sullivan (1943) on hydrocyanic acid production provides a clear-cut demonstration of the physiology of action of complementary factors.

Recently, however, the studies of complementary factors have received accelerated momentum from a different point of view. Dobzhansky (1951) postulated that if a population of genetic constitution *aabb* is divided into two allopatric groups and in one group, *a* mutates to *A* and in another group, *b* mutates to *B*, then these two groups would form local races. These two races can then become sympatric if interbreeding is no more possible because *A* and *B* interact with a lethal effect, thereby resulting in a reproductive isolating mechanism. Thus "the minimum number of genes that can form a workable isolating mechanism is two."

Interspecific hybrid inviability in *Crepis* is caused by a single factor segregating in Mendelian fashion (Hollingshead, 1930). The lethal factor which is ineffective in its own parent species comes from *C. tectorum* and is lethal in hybrids with *C. capillaris, C. bursifolia, C. leontodontoids* and possibly *C. setosa.* The hybrids with *C. taraxafolia* are viable. Furthermore all the strains of *C. tectorum* do not carry this factor; some are homozygous for this lethal, some are heterozygous, while the others are homozygous for its normal allelomorph.

Harland (1915, 1939) first reported that in crossing certain types of Bourbon cotton (*Gossypium hirsutum* var. *marie-galante* Hutchinson) with Sea Island cotton (*G. barbadense* L.) the F_1 hybrids were abnormal, "Streaked with a rough corky outgrowth." Backcrosses of these corky F_1's to *G. barbadense* segregated into 1 Corky : 1 normal from which he suggested that complementary genes were responsible for abnormal cork production.

Stephens (1946) carried out further investigations with the same two species. In crosses, St. Vincent Sea Island (*G. barbadense*) to St. Thomas' Bourbon and Bourbon Intense, both of which are *G. hirsutum* var. *marie-galante,* the F_1 hybrids were corky. The F_2 generation and first backcrosses to Sea Island segregated into 9 : 7 and 1 : 1 ratios, respectively for corky.

The segregating progenies were too small, however, to distinguish between the F_2 9 : 7 ratios expected under complementary factor hypothesis and the F_2 1 : 1 ratios that could result from interallelic interaction at the same locus or interactions of genes at closely linked loci. He concluded by stating "that the complementary allele hypothesis offers a satisfactory interpretataion," and later (1950) hypothesized interaction between pseudoalleles to explain the breeding data.

[1] A part of the thesis submitted to the Graduate Division of the University of California in partial fullfilment of the requirements for the degree of Doctor of Philosophy in Genetics.

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It is interesting to note that only the carriers of this lethal factor (or factors) produce lethal hybrids. An Egyptian type (*G. barbadense*) and Colombian tree-cotton type (*G. hirsutum* var. *marie-galante*) were non-carriers while Sea Island (*G. barbadense*) and St. Thomas' Bourbon, Bourbon Intense (*G. hirsutum* var. *marie-galante*) were the carriers. Of still greater importance is the distribution of carriers. The carrier types are more frequent in the region where the geographical distributions of *marie-galante* and *barbadense* not only overlap, but where the two species have been grown in close contact.

Gerstel (1954) recently reported a new lethal combination in interspecific cotton hybrids. In a series of crosses between species of Asiatic Cotton (*G. arboreum* L., *G. herbaceum* L. and *G. anomalum* Wawra et Peyr.) and new world cotton (*G. hirsutum* and *G. barbadense*) certain crosses produced only lethal F_1 hybrids which are tentatively explained by the hypothesis of pseudoalleles. In each group only certain varieties carry the lethal factor, which by interaction with similar factors from the other group produces lethal hybrids. Thus only Sanguinium (*G. arboreum* L.) carries the lethal factor to interact with that carried by S 5082, S 4025, Coker 100 W, Cemp, Empire and Stoneville (all *G. hirsutum*), the rest being non-carriers.

Hutchinson's (1932) crumpled mutant segregated in F_2 into a ratio of 9 crumpled: 7 normal plants in species crosses. Here also, as in Stephens' and Gerstel's work, only certain varieties and strains carried the factors while others carried their normal allelomorphs.

The object of this paper is to present a clear-cut case of semilethality in species crosses resulting from interaction of complementary factors serving as a reproductive isolating mechanism.

Methods and Material

The stocks were kindly provided by Dr. Charles M. Rick. A stock of the commercial variety Pearson (*Lycopersicon esculentum*) having the genes *sp* (self-pruning) and ms_2 (male-sterile$_2$) markers was used as female parent. *L. hirsutum* form *glabratum* was used as male parent. It was from Dr. Rick's collection from the Rio Pastaza near Baños, Ecuador. This will be referred to hereafter as Baños.

The crosses were made in the field and F_1's raised in the greenhouse for 2½ years. Seeds for F_2 and backcross progeny plantings were obtained by selfing these F_1's and backcrossing them to Pearson. Pearson was used as the female parent. Seedlings of the F_2 and backcross were raised in flats in the greenhouse and then transferred to a cold frame for hardening, after which they were transplanted in the field.

Results

Forty-three F_1 plants that were raised in the greenhouse showed a peculiar behavior of withering in about ten weeks from the date of sowing. The branches would turn pale and droop down as if suffering from drought. The withering continued towards the base. Thereafter, the leaves dried and the whole branch died. Before the whole branch died, however, new shoots were produced from the base which made normal growth for some time. The plants continued to show this behavior at intervals of three and a half to four months.

Table 1. *Segregation of* withering *character in species crosses*, L. esculentum × L. hirsutum *form* glabratum

Withered or withered and died →		Normal	Total	X^2	P
Backcross					
Ob.	192	214	406		
Exp. (1 : 1)	203	203	406	1.19	.3–.2
F_2					
Ob.	125	85	210		
Exp. (9 : 7)	118.1	91.9	210	0.91	.5–.3
Exp. (1 : 1)	105				

None of the plants died in the greenhouse but rooted cuttings taken from these plants and transplanted in the field made some growth and then died. Certain plants of the F_2 and backcrosses grown in the field showed the same behavior. They were classified as shown in table 1.

Discussion

Since neither *L. esculentum* nor Baños is known to show this habit of growth in their natural habitat, or elsewhere, it might be caused—

a. by latent recessive genes maintained in heterozygous conditions in *L. esculentum* and Baños, by some mechanism like balanced lethals, or
b. by the interaction of genes of Baños with the cytoplasm of *L. esculentum* or
c. by the interaction of genes or gene complexes coming from the two parental species.

A. Latent Recessive Genes

The results cannot be explained by balanced lethals or a similar mechanism because no naturally occurring structural hybridity is reported in these species nor is it encountered in the present studies (in press), nor do we have evidence of the presence of lethals in either species. Furthermore, the F_1 consisted entirely of lethal individuals instead of 25% expected on this basis.

B. Interaction of Genes and Cytoplasm

Many cases are known, and others are still being discovered in which certain phenotypes are the result of interaction between cytoplasm of one species and the genes of another species. The work of Renner (1936) on defects of chlorophyll production in species hybrids of Oenothera, of Michaelis (1951) on many characters in Epilobium, and of Jones and Clarke (1943) on male sterility in onions provides us with models of the interaction of cytoplasm of one species with genes of the other. On this basis, the withering of all forty-three F_1 plants and the 1 : 1 ratio observed in the backcross to *L. esculentum* ♀♀ suggest that withering might be caused by the interaction of a single dominant gene from Baños and cytoplasm of *L. esculentum*. But the ratio of 3 withered : 1 normal expected in the F_2 on this basis is significantly different from the observed one (P.001), and the hypothesis is thereby rejected.

C. Interaction of Genes or Gene Complexes

The 1 : 1 segregation observed in the backcross to *L. esculentum* and withering of all forty-three F_1 plants suggest monogenic control of this character, *L. esculentum* carrying the recessive allele and Baños the dominant one. It cannot, however, be under the sole control of a single gene because pure Baños, though homozygous for the dominant allele, did not wither whereas heterozygous F_1's did. This suggests interaction between alleles at one locus. If such is the case, the F_2 progeny should segregate into one-half normal which are heterozygous for this pair of alleles. But the F_2 segregation indicates very strong deviations from this 1 : 1 expected ratio (P.01).

The only remaining explanation of those considered is the complementary interaction of nonallelic genes from both parental species.

We will arbitrarily assume *L. esculentum* to have the *wwSS* genotype and Baños to have *WWss* genotype, the interaction between *W* and *S* producing the withering phenotype. Since both the parental species are homozygous for these genes, all 43 F_1 plants possessed the withering phenotype *WS*. The backcross to *L. esculentum* satisfies the expected ratio of 1 withering (*WsS*-) : 1 normal (*wwS*-). Furthermore on the basis of this complementary interaction the F_2 progeny should segregate in a 9 withered : 7 normal ratio, which too is evidently satisfied.

Both *L. esculentum* and *L. hirsutum f. glabratum* are self compatible; furthermore, there is greater similarity in the morphological characteristics of these two entities than between *L. esculentum* and typical *L. hirsutum*. Similarly, the genetic affinity between these two is stronger than that between *L. esculentum* and typical form of *L. hirsutum* (in press) or any other species of *Eriopersicon* (Rick—oral communication). In addition, though both *esculentum* and *glabratum* are strictly self-pollinating in north temperate regions, the former is known to be considerably cross-pollinated in the native habitat of the genus (Rick, 1950). Under these circumstances of morphological similarity, genetic affinity and provided interspecific cross-pollination occurs in the natural habitat, some type of internal isolating barrier would necessarily be present to keep the species separate. The semilethal complementary factors provide such a mechanism.

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NOTES AND COMMENTS

CATASTROPHISM AND THE FOSSIL RECORD

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Several stimulating contributions from the fertile pen of Otto H. Schindewolf of Tübingen University deal with the enigmatic, apparently world-wide, major interruptions in the fossil record which mark the boundaries of the eras, largest divisions of geologic time (Schindewolf, 1950, 1953, 1954). Abrupt paleontological changes at these stratigraphic levels are real, approximately synchronous, and are recognizable at many places in different parts of the earth where fossiliferous rocks of appropriate ages are represented and have been carefully examined.

Critical events in the history of life evidently were responsible for these world-wide revolutionary changes. With the exception of the lower limit of the Paleozoic, which is marked by the introduction of a varied fauna of shelled marine invertebrates, these stratigraphic levels are characterized by the abrupt dropping out of all of the species, most of the genera, and many of the higher categories (superfamilies, orders and classes) characteristic of the times. The Permian period, at the close of the Paleozoic era, was especially marked by the termination of numerous higher categories of aquatic invertebrates and fishes, many of which had been flourishing throughout the warm shallow epicontinental seas of the world (fig. 1). The extinction, without issue, of many stocks of terrestrial animals and plants also occurred at this time, but on a much more limited scale than among the aquatic forms. Within some of the most complete sequences of non-marine strata (e.g., the Gondwana rocks of the southern hemisphere, or the Permo-Triassic of the Russian Platform), there is no outstanding faunal and floral change that clearly marks the boundary between the Paleozoic and Mesozoic eras (Romer, 1945, p. 531). Consequently the known record of terrestrial life at the Permo-Triassic boundary seems to be more complete in some ways than the record of aquatic life.

The abrupt disappearance at the end of the Permian period of so many characteristic marine forms was followed during the ten million years or so of the early Triassic by depauperate cosmopolitan faunas which only gradually regained part of their former richness by middle Triassic time.[1]

Changes at the close of the Mesozoic era likewise involved sweeping extinctions of diverse marine groups (ammonites, belemnites, rudists, several groups of holostean fishes, marine reptiles, etc.) and the dominant tetrapods of the time, the dinosaurs. As in the Permo-Triassic crisis these late Cretaceous extinctions cut a broad swath across diverse and relatively unrelated ecological situations and differentiation to something like the former diversity was gradual. Most of the orders of early Tertiary mammals did not appear immediately after the extinction of the dinosaurs, but they "straggled in over a span of some twenty million years" (Simpson, p. 365, 1952). This pattern of gradual realization of adaptive opportunities is approximately what one would expect of a few lineages confronted by many newly vacated habitats. Ecologic colonizations were at first slow, then they became very rapid, finally slowing down again as available niches became occupied.

Geologists have long supposed that rates of evolution and extinction are in some manner influenced by the ecological changes induced by orogeny. As long as it was believed that great areas of the crust of the earth were simultaneously disturbed by mountain building, this theory seemed satisfactory. In recent years, however, it has become increasingly evident that orogenic distrubances and associated ecological changes are actually rather restricted in extent and therefore of minor evolutionary importance. This is particularly true with respect to marine organisms. Evolutionary episodes, as revealed in the world record of fossils, apparently do not coincide closely with times of mountain building (Schindewolf, 1950; Henbest *et al.*, 1952).

Paleontological gaps are usually attributed to nondeposition, non-preservation, or to erosional hiatus. In the first case, the time during which neither erosion nor deposition occurs probably is always extremely short. If the sea bottom or overlying waters are habitable by shell-bearing animals, a deposit of shells will inevitably accumulate and may become part of

[1] Schindewolf, 1954, table 1, lists 22 categories of marine and continental animals above the family level that became extinct during or at the close of the late Permian and 11 that appeared as new elements in the early Triassic. The new categories, however, in most cases were represented by primitive forms that had not yet diverged very far from ancestral Paleozoic stocks.

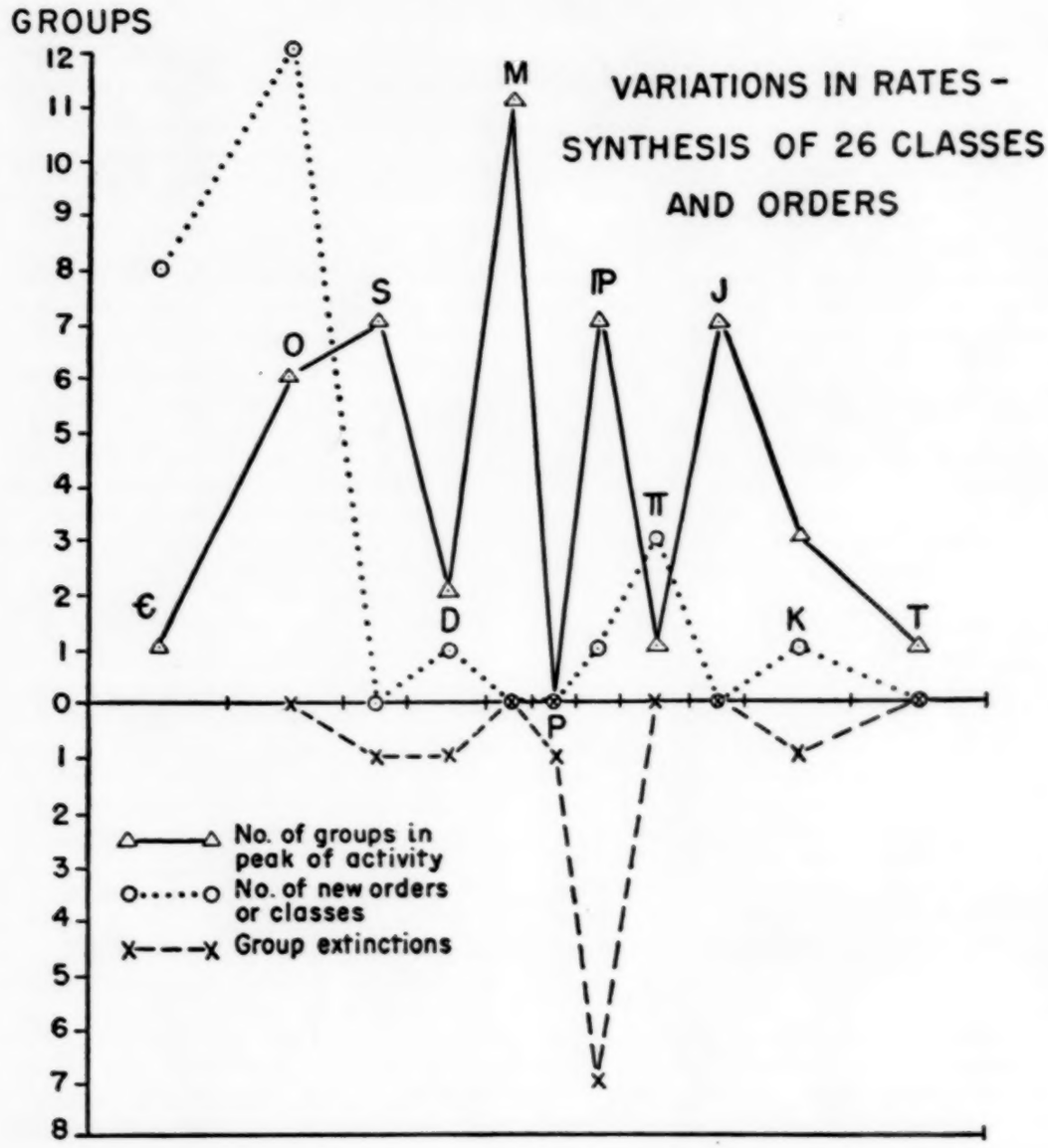

FIG. 1. Rates of evolution and extinction among the best known higher categories of marine invertebrates. Chief times of extinction of orders or classes were the Silurian (S), Devonian (D), Pennsylvanian (P), Permian (IP) and Cretaceous (K), but the Permian was especially outstanding (after Newell, 1952). The proportion of new genera known to appear at any time is taken as a rough measure of evolutionary activity.

the record. Gaps in sedimentation which result from nondeposition (diastems) rarely are recognizable. The record may locally be condensed because of unusually slow rates of deposition (Haas, 1953, p. 302).

On the other hand, erosional unconformities are in many cases easily recognized by evidences of exposure to weathering and erosion by running water. The relative time duration of an unconformity is not revealed by the rocks themselves but must be measured by means of fossils.

If strata below and above an obscure unconformity are parallel, recognition of the erosion surface often depends entirely on paleontological evidence of a hiatus. In the absence of paleontological evidence, continuous sequences of parallel strata are not proof, or even suggestive, of continuous deposition. This has become axiomatic in many quarters, but Schindewolf seems to believe otherwise.

Physical evidence of an unconformity at the era boundaries may be obscure or equivocal at some localities. For example, the Permo-Triassic rocks are approximately parallel over an area of hundreds of thousands of square miles on the Colorado Plateau (Kaibab-Moenkopi formations) and in the northern Rocky Mountains (Phosphoria-Woodside). At many outcrops the contact between the two systems

is structurally concordant and apparently conformable. Regional studies, however, show that an unconformity separates the Permian and Triassic rocks probably throughout all of this region (e.g., Newell and Kummel, 1942). The Permo-Triassic rocks of Greenland, the Dolomite Alps, the Salt Range of Pakistan, and other regions show rock sequences which are not everywhere clearly divided at the systemic boundary by a visible unconformity. Nevertheless, there is a striking paleontological discontinuity, which occurs between the upper Permian and the overlying lower Triassic in all of these areas. This is usually interpreted as indicative of a major stratigraphic hiatus, but Schindewolf interprets it in another way.

Probably the most complete sequence of Permo-Triassic marine faunas thus far intensively studied is found in simple succession in the Salt Range of Pakistan. After an extensive personal examination of these rocks and the contained fossils, Schindewolf draws a number of interesting conclusions with broad implications about the history of life (Schindewolf, 1953).

Schindewolf confirms reports by numerous investigators that there is no evident physical break between the Permian and Triassic rocks in the Salt Range. As a further indication of stratigraphic continuity he calls attention to an ancestor-descendent relationship in the evolution of one of the cephalopod lineages. This example is almost unique among the ammonoids, however, and it serves to emphasize the fact that an overwhelming majority of Permian lineages did not leave Triassic descendants.

Neverthless a striking faunal change separates the two systems. As in all other studied sequences, not a single species crosses from the Permian to the Triassic and only a small percentage of the genera are common to the two systems. Many superfamilies, orders and classes of Permian invertebrates drop out abruptly at the Permo-Triassic boundary. Most of the genera and many of the families of lower Triassic invertebrates are not known to occur in the Permian rocks. It seems to Schindewolf that an explanation of this situation must include catastropic environmental changes leading to termination of many lineages, and to sudden divergence of surviving stocks to form a wholly new fauna.

According to Schindewolf the most likely cause of this and other remarkable interruptions in the fossil record, at the base of the Paleozoic, and at the top of the Mesozoic, is some unknown extra-terrestrial agency, such as a powerful burst of solar or cosmic radiation. He believes that such a catastrophe might simultaneously produce extinctions in many groups and accelerated mutation rates in others, thus leading to unusually rapid differentiation and replacement. Known geological processes, such as changes in distribution of land and sea, he believes, are much too slow to produce the observed results. These conclusions of Schindewolf represent a considered attempt to explain geological phenomena that are extremely controversial and puzzling. They harmonize well with his faith in the theory of macromutation.

The suggestion that the Permo-Triassic sequence in the Salt Range represents continuous sedimentation hardly seems justified to me from the evidence at hand. The situation is very like that of the much more wide-spread rocks of the western United States already mentioned where for many years an important unconformity between Permian and Triassic rocks was not recognized. Before their fossils were evaluated, rocks now regarded as lower Triassic in age were, in many areas, classed with the Carboniferous or Permian.

Schindewolf's views about the Permo-Triassic boundary evidently are influenced by his belief that the fossil record of marine invertebrates is relatively complete. As evidence of this, mutually similar or identical and synchronous sequences of ammonoids and graptolites in many places are cited. These sequences, he believes, would not match one another so closely if incompleteness of the fossil record were the rule (Schindewolf, 1950, p. 33). It seems to me, however, that he is overly impressed by these selected examples of matching fossil sequences and it is not difficult to find exceptions to his generalization. Many sequences of a given age show divergent paleontological characteristics for each one that approximates any given standard.

It seems probable that the sharpness of the paleontological break between the Permian and Triassic, which characterizes even the most complete sequences known, argues for an unusually wide-spread stratigraphic gap (on the continents but probably not under the oceans) of some millions of years duration, or sufficient time for the Permian marine faunas to be replaced by different Triassic faunas.

Schindewolf's assumption that Permo-Triassic deposition was continuous in the Salt Range complicates the problem and requires an unnecessarily involved explanation for the great paleontological break. His view leads, of necessity, to the conclusion that some unusual, catastrophic [2] process was responsible for the observed relationships.

We can agree with him that known geologic (and biologic) processes probably would not

[2] Schindewolf (1954, p. 454) feels that he is really not appealing to catastrophe, but this is a matter of semantics. It seems to me that the facts here are marshalled to fit the theory instead of vice versa.

suddenly produce sweeping and world-wide changes in the known marine faunas at the close of the Paleozoic. It does not follow, however, that the hypothetical cosmic agencies, suggested by Schindewolf, offer a more satisfactory solution to the problem than demonstrable geologic and biologic processes. It seems to me more in keeping with time-tested scientific procedure to avoid, if there is a practical alternative, hypothetical solutions, no matter how tempting, that depend on highly speculative and untested premises.

It seems highly improbable that sudden large doses of cosmic or solar radiation could produce the observed effects. Intensities of ultra-violet light or cosmic rays great enough to be lethal to terrestrial organism of ordinary tolerance probably would not affect aquatic organisms at depths of a few meters. Water is a particularly effective filter of radiant energy of both short and long wave lengths (Clarke, 1939). The evolutionary effects should be, according to Schindewolf's theory, overwhelmingly greater among terrestrial than among aquatic organisms. This is not the case.

Although evolutionary activity among marine invertebrates was high in middle and late Triassic times, there is little indication that it was really exceptional during the early Triassic, although many higher categories apparently originated at this time. His assumption that accelerated mutation rates would necessarily result in increased evolutionary activity is also certainly doubtful. It has been sufficiently demonstrated that the vast majority of mutations are deleterious. Furthermore, there is some indication that natural selection actually curbs mutation rates. A sudden flood of "hopeful monsters" within a population could not but have effects unfavorable for the race as a whole. Survival and the rate of spread of new genes throughout a population would be determined mainly by natural selection rather than by frequency of mutation.

Schindewolf believes that the best way to explain many of the innumerable small and the few large discontinuities in the fossil record, some of which he thinks were never completely filled by transitional forms, is by means of catastrophic extinction and simultaneous creation of new faunas. They are more simply and accurately explained, I believe, as stratigraphic breaks, many of which are recognizable and evaluated solely by paleontologic criteria. These breaks punctuate a record of gradual, although not uniform, evolutionary change. The gaps may be caused by local irregularities in the distribution of once living populations, to non-burial, non-preservation, and to subsequent erosion of the record. They commonly correspond to the early, sometimes rapidly evolving, phase of lineages when populations are very small and probabilities of discovery are correspondingly low.

The majority of species of living marine invertebrates live in and are restricted to warm shallow waters, and the fossil record suggests that this has always been so. Furthermore, the great dispersal centers probably have always been the epicontinental seas which just now appear to be greatly restricted. Many species have been adapted to, and probably even confined to, landlocked seas. Subsidence of the oceans at the present time by little more than one hundred fathoms would cause withdrawal of the shelf seas from the continents and the elimination of many habitats with consequent extinction of many species. Particularly revolutionary must have been the changes in shallow marine conditions at the close of the Permian period as the sea waters withdrew for a time into the ocean basins. The physical evidence is reasonably convincing that the epicontinental seas were completely drained at this time.

In opposition to this view it might be argued that drastic reduction in numbers of the natural populations of the epicontinental seas by universal (eustatic) drop in sea level would be followed by migration and colonization of the newly shallow waters along the continental slope. Depth of water, however, is only one of the many factors. It seems very doubtful that many of the ecological conditions of shallow inland seas could be reproduced on a steeply sloping bottom facing the open ocean.

It is well established that reduction of a natural population below a critical size, from whatever cause, commonly results in an excess of deaths over births.[3] Organisms in many ways tend to improve their local environment. Optimum population density often makes animals less susceptible to toxic substances, lack of oxygen, extreme temperatures and other hazards than does under-population (Clarke, 1954). Reduction of normal population density greatly diminishes the chances of reproduction in all bisexual animals, but this becomes indeed crucial among those aquatic forms in which the chance union of gametes varies logarithmically with population density. Thus, the final extinction of a species may be brought about by intrinsic factors within a population after it has suffered severe reduction in number from extrinsic causes. The most obvious extrinsic factor which has been in operation throughout known geologic history is world-wide (eustatic) changes in sea level. These

[3] The principle of the minimum population.

changes have had profound effects on shallow-water marine organisms. During maximum flooding of the continents, as during the Ordovician, Mississippean and Cretaceous periods, the total area occupied by shallow seas was thousands of times greater and correspondingly more diversified than at times of maximum emergence of the continents, as the interval between the Permian and Triassic periods. These fluctuations of sea level by a few hundred feet also have greatly affected the nature and scope of terrestrial habitats with consequent wholesale evolutionary effects.

The sinking of the ocean basins and ultimate drainage of the inland seas probably has always been slow. But the epicontinental seas and their manifold habitats have, of course, always been sensitive to eustatic changes in sea level brought about by earthly phenomena, such as continental glaciation and diastrophism within the ocean basins. Examination of the record of marine fossils shows considerable correspondence, not with times of mountain building, but with times of great world-wide innundation and drainage of the continents (e.g., Cooper and Williams, 1952; Moore, 1952).

This is the traditional explanation of interruptions in the record of marine fossils and it satisfactorily explains many of the observed conditions in the stratigraphic succession. It is not certain that changes in sea level have caused simultaneous extinctions of major groups of marine and terrestrial animals. And it is not at all certain that these have had a common external origin. Perhaps it is futile to search for a single cause for all of the great mass extinctions. Since the Permo-Triassic crisis did not involve terrestrial animals to the same degree as that at the close of the Mesozoic era, the two may have resulted from somewhat different causes.

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ADAPTATION OF MAN TO COLD CLIMATES

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The purpose of this note is to agree broadly with Scholander's views (this journal, Vol. IX, No. 1, 1955) on the adaptation of homeotherms to cold climates, and to outline the bodily adaptations of man in the arctic that were not mentioned. Scholander is certainly correct that decrease of heat dissipation in man as in other homeotherms plays the major role in adaptation to cold. While heavy insulation in the form of fur is obviously no phylogenetic adaptation for man, specially designed fur clothing and some efficient means of shelter are the two prime factors permitting man's late Pleistocene shift from a warm Pontian to a cold Holarctic fauna. Indeed it seems likely that the clo

$$\left(\frac{1 \text{ clo} = 0.18^\circ \text{ C.}}{\text{cal./sq.m./hr.}}\right)$$

units of native attire closely parallel the insulating values of winter fur cited in Scholander's figure 2.

In view of man's relatively recent emergence

from the tropics, and because of his cultural accoutrements to cope with cold, the efficient vascular control of the less well insulated extremities seen in arctic fauna cannot be matched by humans. Nevertheless man shows some adjustments in this direction. While some cold climate mammals and birds can maintain leg temperatures at 10° C. and lower (Scholander, 1955, p. 19), tests on American Whites show that under more moderate cold at 15° C., the surface of the feet can remain without apparent injury at 17° C. for a month (Spealman, 1949, p. 236). Possibly cold-adapted people like the Eskimos could maintain lower temperatures in their extremities, but this has not been tested. Clearly the Eskimos have not achieved full physiological adaptation to cold. To cite two sorts of evidence, the most common operation performed on Alaskan Eskimos is said to be amputation of frozen toes (Coon, Garn, and Birdsell, 1950, p. 43).

While Scholander is largely correct when he says (p. 23), "In the Eskimo the main adaptation lies not in the physiology, but in an age-long experience and technical skill in ducking the cold," in the long view he has underplayed two factors. First, the technical skill in ducking the cold probably resulted only from long-term experimentation, and during this time the cold would have a more drastic screening effect upon the experimenters. Secondly, while Eskimos surround ". . . themselves with a little piece of the same tropical microclimate upon which we also depend" (p. 23), this is contingent upon plentiful supplies of high calorie food. Since the Eskimo winter house is heated largely by the bodies of its inhabitants, up to around 60° F. with possibly a 100° F. indoor-outdoor gradient (Stefansson, 1955, p. 36), these radiators need constant stoking. An Eskimo outdoors, though clad in a cold resistant double garment of as much as 4 clo in insulating value, needs food to keep warm enough for effective hunting. Any doubt that short supply and starvation are the principal hazards of Eskimo life may be dispelled by the many references listed by Weyer (1932, pp. 116–117 ff.). It seems likely that Scholander has also underplayed the apparent physiological superiority under cold stressing that Eskimos show over Whites, and particularly Negroes (Meehan, 1955, pp. 333–334). The higher surface temperatures maintained by Eskimos whose fingers are immersed in an ice bath may be, as Scholander (p. 23) notes, a heat-wasting tropical type of emergency reaction. These higher finger temperatures corroborate Brown and Page's (1952, p. 229) data on the greater blood flow in Eskimo than in Whites hands during an ice bath. Yet heat-wasting emergency reaction or not, it seems clear that the ability to maintain sufficient extremity temperatures to prevent freezing has selective value in cold climates. Military annals provide practical demonstrations of this, with the higher incidence of cold injuries in Negro than in White troops during the Korean winter of 1950–51, and the two subsequent winters at Ladd Air Force Base, Alaska (Meehan, 1955, p. 330).

Similar finger-ice bath experiments were conducted in Manchuria, where the highest temperatures were maintained by a series of Tungus-speaking mountain people, the Orochons (Yoshimura and Iida, 1952, p. 179). Mongols and Chinese ranked together, and the Japanese then living in Manchuria showed the lowest temperatures. Furthermore, Manchurian-born Chinese showed higher finger temperatures than those born in Shantung, and Japanese from the colder areas of their home islands had higher figures than those from the warmer areas. It is likely that in both the Eskimo-White-Negro and the Orochon-Mongol-Chinese-Japanese studies acclimatization during the individuals' life span and racial heredity served as complementing factors. In addition, Coffey (1955) found manual dexterity under arctic conditions to be very superior in young Eskimo and Indian men as compared to acclimatized Whites.

Sargent (1953) has provided a summary of our physiological knowledge of short-term acclimatization in Whites. With the pituitary-adrenal system as the controlling mechanism, a reactive syndrome of changes occur. Some of these are vasoconstriction and shifting of the blood from superficial areas to the pulmonary and probably splancnic regions, reduced skin temperatures, blood plasma diminution, hemoconcentration, and metabolic increases.

Whether these short-term changes are similar to the physiological patterns of Eskimos and other arctic peoples has not been determined. The decrease of blood volume in Southampton Island Eskimos during the summer months (Brown et. al. 1954, p. 254) seems antithetical to the White syndrome. Yet a similarity is suggested by Roberts' (1952 a and b) findings that among native peoples, including those from the arctic, basal metabolic rates and blood pressures seem to rise with lowered mean annual temperatures, while pulse and respiration rates are lowered. Possibly the higher blood pressures of cold climate peoples are principally a reflection of vasoconstriction, but here some proof is needed.

Basal metabolic rates of Eskimos are consistently high, ranging in means from +12 to +33 during the wintertime (Bollerud, Edwards, and Blakely, 1950, pp. 1–2). Repeated determinations on the same Southampton Island Eskimos under strictly basal conditions gave high BMRs, although about a 6 per cent drop occurred during the summertime (Brown et. al. 1954, p. 253). The reasons for the higher basal

metabolic rates in Eskimos are not wholly clear. Rodahl (1952) made 340 tests on 73 healthy Eskimos, and found that while subsisting on native food their BMRs averaged +8% over White norms. When they changed to a White diet, their BMRs fell to an average of −8%. This should indicate that the significantly higher basal metabolic rates of Eskimos are largely dietary in origin. In connection with U. S. Military personnel in the arctic, Gray, Consolazio, and Kark (1947) have evidence that the metabolic increase due to the hobbling effect of clothing on military personnel seems at least twice as great as that due to lowered temperature. In this connection Stefansson (1955, p. 51) states that Eskimo winter clothing only weighs 7–10 pounds, as compared to 15–25 pounds for the usual military arctic gear, and would, I assume, have a lessened hobbling effect. Another explanation for the higher metabolic rates and caloric intake in the arctic comes from Webster (1952, p. 134), who views them as compensation for the considerable heat lost in warming and humidifying inspired air to body temperature.

In addition to calorie increase as a subsidiary means of adjusting to the cold, experiments show (Swift and French, 1954, p. 221) that high-fat diets are superior in maintaining tissue temperature to high carbohydrate and especially high protein diets. Heinbecker (1928, p. 474 ff.) speaks of the Eskimos' remarkable capacity to oxidize the plentiful fats in their diet. In explaining the superiority of high fat diets, Swift and French (idem) suggest that it is more concerned with reducing heat dissipation than increasing heat production, and ". . . may involve a temporary deposition of dietary fat in the subdermal tissues following a high-fat meal." No proper measure has been made of the subcutaneous fat covering of people like the Eskimo, but it is generally assured to be thicker than in temperate climate people. If this is actually true, the facts that subcutaneous fat has insulating qualities ⅛ to ½ better than muscle (Edholm, 1954, p. 210), and that 1 cm of fat has a 1 clo insulating rating (Bazett, 1949, p. 145), should give Eskimos a clear adaptative advantage.

Besides these physiological alterations contributing to withstanding the cold, there are significant morphological changes as well. While the causal relationship between the body mass/body surface ration and climate has not been demonstrated, the correlation between the two cannot be denied for man. Roberts (1953, p. 542) found a correlation of −0.60 between mean body weights in 116 native groups, including 11 from the arctic, and mean annual temperatures of their locations. The range of means ran from about 42 to 72 kg, and there was an average increase of .305 kg for 1° F. decrease. Corroboration of Roberts' work comes from R. W. Newman and Munro's (1955, p. 10) determination of a correlation of −0.611 between body weight and estimated skin surface in 15,000 U. S. Army inductees with the mean January temperature of their state of birth. Here the regression is .109 kg increase with 1° F. decrease. Then R. W. Newman (1955) found high negative correlations in U. S. troops between (1) fat free or lean body weights and mean January temperatures of their birth areas, and (2) subcutaneous fat and mean July temperatures of their birth areas. These findings suggest that in colder climates the proportion of metabolically active tissue to inert tissue is higher than in warm climates.

Additional confirmation comes from Schreider's (1951) demonstration that mean body weight/body surface rations run about 38 in colder climates, while in hot climates minimum means of 30 kg/m^2 are attained. Since he used the Du Bois height-weight formula which tends to overestimate body surface in cold climate peoples and to underestimate it in hot climate peoples, his mean ranges may actually be slightly extended over those stated. The body size trends in these several studies support the view that Bergmann's rule is operative in man, and in civilized man at that.

Allen's rule has not had such extensive testing in man. There is some evidence in New World aborigines (Newman, 1953, maps 2 and 3) and in the Old World (Lundman, 1952, K.3) that cold climate peoples have relatively longer trunks and shorter legs than others. R. W. Newman's (1953) clothing study on 2,450 Koreans clearly demonstrates their longer trunks and shorter legs as compared with American Whites. Little or no shortening of the arms, however, is apparent in cold climate peoples, whose arm/stature ratios run about one index unit lower than Europeans. Whereas the feet of cold climate peoples are said to be small (Coon, 1954, p. 292), Rodahl's (1952, p. 424) skin surface study on Eskimos shows their hands to be relatively large. Perhaps the arms and hands of arctic peoples serve, when needed, as a means of heat dissipation, as do the flippers of seals and penguins (Scholander, 1955, p. 19 and 24).

The so-called "climatic engineering" of the Mongoloid face—involving reduction of brow-ridges with their cold-vulnerable sinuses, orbital and malar adjustments to permit more fat padding, and reduction of nasal saliency (Coon, Garn, and Birdsell, 1950, pp. 67–71)—is a model of morphological logic, but needs testing. Some data on the superior fat padding of the eye-ball in Chinese is provided by Wen (1934). The reduced beard and beard growth of Mongoloid peoples (Coon, Garn, and Birdsell, 1950, p. 72) may also be a cold weather adaptation

since in cold the exhaled breath will freeze in beards, along with the skin underneath.

All these data should make it clear why anthropologists have paid serious attention to the application of the ecological rules to man. If anything, these rules seem to be more closely operative in man than in other species of homeotherms (see Newman, M. T., 1953, pp. 312–313). Basic experiments on human subjects are proceeding apace, with the view of tying in morphology to physiology, and the two to the environmental setting. Although within the total environment, anthropologists have overemphasized temperature and humidity, they are well aware that other less readily measurable factors such as nutrition and workload may be equally significant. In researches upon human adaptation, perhaps the most pressing need is some means of differentiating between the short-term non-genic alterations and the long-term phylogenetic changes. As Scholander infers (p. 17), this means more and better controlled studies on native peoples.

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GEOGRAPHICAL CHARACTER GRADIENTS AND CLIMATIC ADAPTATION

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The recent discussion by Scholander (1955) of the so-called "ecological rules" indicates a number of misunderstandings. It might be worthwhile to scrutinize the meaning and validity of these rules in some detail, since some of these misunderstandings seem to be based on widespread misconceptions, to judge from discussions with various biologists. Before the discussion a word should be said on terminology. There is no good, generally accepted collective term available for such rules as Bergmann's rule, Allen's rule, etc. The terms "climatic rules" and "ecological rules" have been used, both being rather broader than justified by the phenomena which they describe. The term ecogeographical rules, although by no means ideal, will be used in the subsequent discussions, being less inclusive than the term ecological rules.

What do the ecogeographical rules signify?—They are purely empirical generalizations describing parallelisms between morphological variation and physiogeographic features. For instance, Bergmann's rule states that "Races of warm blooded vertebrates from cooler climates tend to be larger than races of the same species from warmer climates." As Rensch has emphasized consistently, this is a purely empirical finding which can be proven or disproven no matter to what physiological theory one might ascribe this size trend. The validity of an ecological rule then does not depend on the validity of the physiological interpretation, but merely on the reliability of the empirical finding. To prove that an ecological rule is invalid one would have to prove that it is not valid in the majority of relevant cases. For instance, one would have to prove that races of warm blooded vertebrates in cooler climates do not tend to be of larger average size than races in warmer climates.

This clear separation between empirical findings and interpretation has not always been observed by some of the early authors (e.g., Bergmann) or in some of the textbooks. It is therefore necessary to emphasize once more that two independent steps are involved: first, the establishment of a regularity (and these regularities may be very different in mammals, fishes, or insects) and second, the physiological interpretation given to this regularity.

Laws or rules? A few authors, mostly those without first-hand experience with the phenomenon, have assumed that these regularities are "laws" that are invariably true. However, right from the beginning authors like Gloger (1833) and Bergmann (1849) have stressed that these rules have only statistical validity. They are true for "most" species or "many" races. Rensch in particular has emphasized this point and has devoted a series of papers (Rensch, 1936, 1938, 1939, 1940, 1948) to a determination of the percentage of cases in which the stated regularities have validity. These rules work only "other things being equal." The principal object of Rensch's investigation was not so much to prove the climatic rules, but rather to determine the degree of their validity for different kinds of animals in different regions.

Do the ecogeographical rules apply to species as well as to races? Bergmann and other early authors applied these rules equally to species and races, but it must be admitted that most of their "species" are considered geographic races by modern authors. It is true that these rules are sometimes valid on the species level, and Hesse, Allee, and Schmidt (1951) have listed a number of such cases. Yet nearly all contemporary supporters of the ecogeographical rules have pointed out carefully that they have validity only for populations within species.

The general viewpoint concerning the limits in the application of the ecogeographical rules has been stated by Mayr (1954) as follows: "It has long been known that the validity of the so-called ecological rules, so far as it exists at all, is restricted to intraspecific variation. . . . A more northerly species is by no means always larger than its nearest more southern relative." The fact that "cold climates do not produce a fauna tending towards large-sized globular forms with small protruding parts" (Scholander, 1955) is not in the least in conflict with Bergmann's or Allen's rule.

The physiological interpretation of the ecogeographical rules. A number of recent authors have stressed that there is a difference, at least in degree if not in kind, between the adaptation of a population to local conditions and the adaptation of a new kind of animals or plants to a novel ecological niche. Adaptation to local conditions has been referred to as ecotypic adaptation (Turesson) or existential adaptation (Goldschmidt). It is quite evident that the ecogeographical rules formulate empirical findings concerning the local type of adaptation. They have never been interpreted by evolutionists as playing "a detectable role in the phylogenetic engineering of warm-blooded animals for hot and cold climates" (Scholander, l.c., p. 22). Nor have evolutionists contended that the ecological rules "reflect phylogenetic pathways of heat conserving adaptation" (l.c., p. 24). They are an ecotypic, not a phylogenetic phenomenon.

The physiological interpretation will be different on the whole for each ecogeographical rule, depending on whether it concerns size, proportions or coloration, warm-blooded or cold-blooded animals. All interpretations, however, have in common the endeavor to establish a correlation between a character gradient and a gradient in the environment. Rensch's (1939) investigation of the increase in size in *Parus montanus* in correlation with temperature gradients may be selected as an example of this method. A first attempt to correlate the size gradient within the European range of this species with the annual isotherms was unsuccessful. However, when the isotherms of the coldest month were taken, which in Europe is January, they were found to parallel closely the size cline in *P. montanus*. Similarly ichthyologists have established for fishes a correlation between decreasing water temperatures and a tendency toward an increase in certain meristic elements. A close parallelism between character gradients and environmental gradients, if found to have a genetic basis, is interpreted with good reason as being the result of natural selection.

If we take specifically the case of Bergmann's rule, it is entirely consistent with the known facts to assume that the parallelism between temperature gradient and size gradient is correlated with the change in the body surface–volume ratio which is the consequence of a simple law of geometry. The explanation then that the increase of size to the north serves heat conservation and that the decrease of size to the south facilitates heat dissipation is entirely consistent with all the known facts. The hypothesis then that Bergmann's rule is the result of natural selection in favor of an optimal surface to volume ratio is a legitimate one. It is axiomatic in scientific methodology that a hypothesis is considered valid until it has either been disproven or until a better one has been proposed.

The role of exceptions. The need for heat conservation is only one of many possible selection pressures affecting absolute or relative body size. It is to be expected that under certain conditions other factors may affect geographic variation in size more strongly than the need for heat conservation. For instance, if a small mammal lives in burrows during the winter, selection pressure for heat conservation will be greatly lessened and there may be no size increase toward the north. Indeed since an increase in size results in an increased demand for food, size may actually decrease to the north where food is the limiting factor. This was indeed demonstrated by Stein (1951) for the European mole (*Talpa europaea*). The phenotype of an animal is the result of a compromise between many conflicting selection pressures. Every exception to the ecogeographical rules is an indication of such a conflict. These rules are now sufficiently firmly established so that the emphasis of research should be shifted to a study of the exceptions. Snow's (1954) study of trends of geographic variation in palearctic titmice illuminates the reason for some exceptions in this family. Relative bill size, for instance, decreases rapidly northward in these titmice until a minimum size is reached. It appears that the size and the amount of food taken by these birds precludes a further reduction in bill size. This minimum size then is the ultimate compromise of conflicting selection pressures. Exceptions to the rules in other groups of animals should be analyzed in a similar manner.

Length of bird wing and Bergmann's rule. In all studies on the validity of Bergmann's rule for birds the length of the wing is used as an indication of body size. Scholander (l.c., p. 21) criticizes the use of this measurement by Rensch because "there are a few short finger bones in this measurement, the rest is just wing feathers. These measurements correlate with flight, but bear no useful thermal significance." He does no appear to realize that Rensch has nowhere treated the wing as a heat radiating body part. The wing is used as an indication of general body size, in the absence of a better

measure, since "wing load" necessitates a fairly close correlation between wing length and body weight, and the latter is an indication of general size (Amadon, 1943). It is unknown and badly in need of investigation how close this correlation is. A number of studies which have revealed exceptions are already available. It has been pointed out by several authors, including Rensch himself, that a new variable is introduced if northerly populations of an otherwise sedentary species are migratory. In most cases this leads to an attenuation and considerable lengthening of the wing of the northern population. The better a flyer the particular species is, however, the less effect the annual migration will have on wing shape and wing size, and the fact that the populations will spend both summer and winter in a warm climate will become the dominating factor. In *Dicrurus leucophaeus,* for instance, wing length in the highly migratory race of North China (*leucogenis*) (adult males av. 142.8) is no greater than in the more or less sedentary race of South China (*salangensis*) (adult males av. 143.0) (Mayr and Vaurie, 1948).

Special uses of the wing in courtship may set up a new selection pressure which entirely upsets the need for a "normal" wing load. Adult males of the Regent Bower Bird (*Sericulus chrysocephalus*) have a wing which averages 5 mm and a tail which averages 15 mm shorter than that of immature males (Mayr and Jennings, 1952). In the African weaver, *Euplectes hordaceus,* likewise the wing is much shorter in the nuptial plumage (76.3) than in the eclipse plumage (82.7) (Verheyen, 1953).

These findings again are evidence of the multiplicity of selection pressures to which an organ is exposed and of which the final phenotype is a compromise.

Does the existence of other heat conserving mechanisms invalidate the ecogeographical rules? Scholander's criticism of the physiological interpretataion of Bergmann's and Allen's rules is based on the fact that other heat conserving mechanisms, such as an increased density of fur or plumage, fat deposits, or vascular mechanisms, are immeasurably more efficient than the slight shifts in surface to volume ratios demonstrated by the ecological rules. The facts of physiological adaptation to life in a cold climate discovered by Irving, Scholander, and other recent investigators are of the greatest interest and can be considered as unequivocally established. Yet they do not permit the conclusions drawn by Scholander.

The philosophy of "all or none" solutions is exceedingly widespread not only in science but in all human affairs. Unfortunately no philosophy could be worse suited for evolutionary studies. All or none solutions are based on typological thinking and alien to the facts of variation. Multiple solutions for biological needs are the general rule in evolution. An animal is protected against a predator not by speed *or* an armor *or* by cryptic coloration *or* poison *or* bad taste *or* by hiding *or* by nocturnal habits, but always by a combination of several of these. Simple answers are nearly always misleading. It took ornithologists a long time before they got away from such shortsighted single aspect statements as "birds start breeding in spring because of increasing day length," or "birds migrate in fall because of a drop in temperature." Does the fact that a thicker fur or denser plumage increases protection against the cold completely eliminate any selective advantage of an improved body surface to volume ratio? Surely not!

The true meaning of geographical character gradients. The regularities in the geographic variation of general size and of proportions are a simple matter of fact, as pointed out above. Vaurie, for instance, showed that all species of drongos show variation of wing length correlated with latitude except those restricted to a single island or those that live in a single restricted climatic zone (Mayr and Vaurie, 1948). Adherence to the ecological rules is, however, usually not found in the case of isolated populations (Mayr, 1942), a fact which is an important clue in the solution of this problem. It is becoming increasingly evident that such ecotypic adaptations as manifest themselves in the ecological rules are the means of a local population for reaching a balance between (1) the need for adaptedness to local conditions and (2) partaking at the same time of the heritage of the species as a whole, which includes all physiological mechanisms that are species specific. A species is a single large Mendelian population pervaded in all directions by gene flow. It is this cohesive force of gene flow which is primarily responsible for the validity of the climatic rules. It gives physiological unity to a species but increases the necessity for local adjustments to local conditions. That these local adjustments, reflected in the ecological rules, have only limited effectiveness is evident from the fact that even the species which obey the rules find northern and southern limits to their geographic range.

The sensitivity of this process of local adaptation is evidence of the universality of natural selection and this is the crucial point of the entire question. Even genes with a selective advantage of only a fraction of one percent tend to accumulate in populations, as shown by R. A. Fisher (1930) and others. Natural selection is particularly efficient during catastrophes and other periods of great environmental stress. Let us look at a model of the effect of such a catastrophe on the survival of two alternate mechanisms adding to the survival value. Let us assume that in a population of mammals, which is at rare intervals exposed to very se-

vere winter conditions, there are 10 individuals with a superior vascular heat conserving mechanism and also 10 with a greatly reduced tail length among the total population of 1,000 individuals. After a severe winter the 10 individuals with the superior vascular mechanism might survive as well as two with the greatly reduced tail length. After an even more severe winter only one individual might survive which happens to have the combination of the superior vascular mechanism and the reduced length of tail. Students of natural selection know that such a model is quite realistic and that genes accumulate in a population, independently of each other, in accordance with the contribution they make to fitness. Problems of selection are simply statistical problems and the prevailing phenotype of a population will be the result of a balance between opposing selection pressures. This is the meaning of the ecogeographical rules, nothing more and nothing less.

It appears to me that there is no contradiction between Scholander's interesting findings on the heat preserving adaptations of arctic species of birds and mammals, and rules like Bergmann's and Allen's rules, which deal with adaptation of intraspecific populations to local conditions. Either set of factors does not disprove the other.

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